Targeting the Liver via the Asialoglycoprotein-Receptor:

Synthesis of Directed Small Molecule Libraries for the H1-CRD.

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Claudia Riva

aus Montevideo, Uruguay

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Referent: Prof. Dr. Beat Ernst

Coreferent: Dr. Eric Francotte

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von

Referent: Prof. Dr. Beat Ernst

Coreferent: Dr. Eric Francotte

Vorsitz: Prof. Dr. Matthias Hamburger

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Prof. Dr. Hans-Jakob Wirz

Dekan

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Declaration

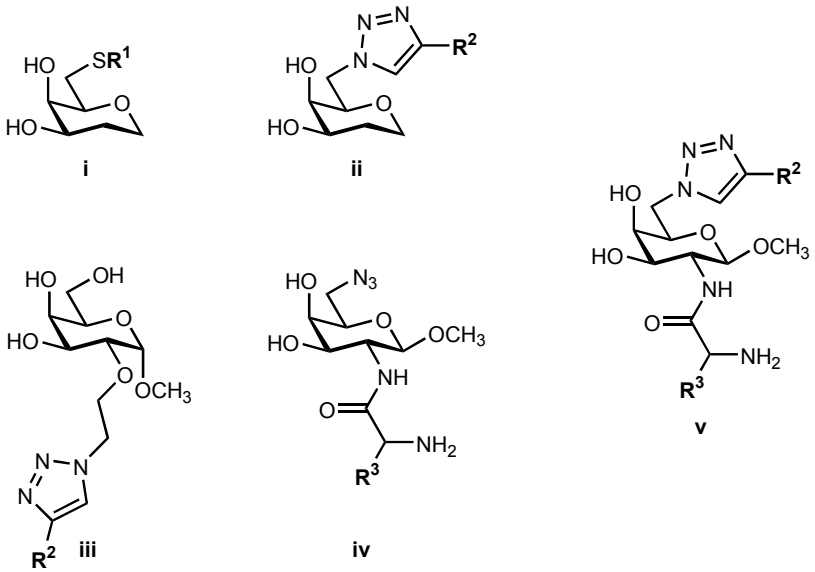
I declare, that I wrote this thesis (Targeting the Liver via the Asialoglycoprotein-Receptor: Synthesis of Directed Small Molecule Libraries for the H1-CRD) with the help indicated and only handed it in to the Faculty of Science of the University of Basel and to no other faculty and no other university.

Claudia Riva
Basel, January 2006

Abstract

Asialoglycoprotein-receptors (ASGP-Rs), exclusively located on the cell surfaces of hepatocytes, bind terminal galactose/*N*-acetylgalactosamine residues of desialylated glycoproteins and mediate their endocytosis. Galactosyl-containing molecules can therefore be employed as hepatotropic vectors of drugs for site-specific drug-delivery via the ASGP-R. Studies examining the structural requirements of simple monosaccharide derivatives for high affinity binding to the human hepatic lectin were performed. The affinity of various synthetic *galacto*-derivatives was assessed by a competitive target-based assay and surface plasmon resonance experiments using the carbohydrate recognition domains (CRDs) of the H1-subunit of the ASGP-R. This work provides experimental data for simple synthetic ligands for the human H1-CRD.

Based on the crystal structure of the H1-CRD and SAR studies for similar receptors, four families of D-galactose and D-galactosamine derivatives and mimics thereof, modified at the 2- (**iii** and **iv**) or 6-position (**i** and **ii**), were synthesized either in solution or on solid phase. Similar or improved affinities relative to Gal*N*Ac were obtained. Substituents in the 2-position (**iii** and **iv**) tightly interact with the receptor, while substituents in the 6-position (**i** and **ii**) could only modestly improve the affinity. However, with di-substituted monosaccharide mimics **v** the additivity of the effects of substituents in the 2- and 6-position could not be confirmed.



R^1: hydrophobic groups, **R**^{2}: hydrophobic or hydrophilic groups **R**^{3}: H, CH_3 or $CH(CH_3)_2$

Abbreviations

Ac	acetyl
AcOH	acetic acid
Anal	analitycal
Aq	aqueous
ASGP-R	asialoglycoprotein-receptor
ASGPs	asialoglycoproteins
Bn	benzyl
BSA	bovine serum albumin
Bz	benzoyl
calcd	calculated
CRD	carbohydrate recognition domain
CSA	camphor sulfonic acid
d	days
DABCO	1,4-diazabicyclo [2.2.2] octane
DBN	1,5-Diazobicyclo [4.3.0] non-5ene
DCE	dichloroethane
DCM	dichloromethane
DMSO	dimethylsulphoxide
DEAD	diethyl azodicarboxylate
DIC	*N,N*'-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N,N'*-dimethylformamide
EA	ethylacetate
ELS	emitted light scattoring
eq	equivalent
ESI	electrospray ionization
Gal	galactose
Gal*N*Ac	*N*-acetylgalactosamine
Glc	glucose
h	hours
H1	major subunit of the human ASGP-R

H1-CRD	carbohydrate recognition domain of the major subunit of the human ASGP-R
H2	minor subunit of the human ASGP-R
HBTU	2-(1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HBV	hepatitis B virus
HCV	hepatitis C virus
HOBt	hydrate-1-hydroxybenzotriazole
HPLC	high performance/ pressure liquid chromatography
IC_{50}	concentration of ligand required for 50% of inhibition
(IFN)-α	interferon
IgA	immunoglobulin A
IR	infra red
K_D	equilibrium dissociation constant
KDa	kilo dalton
LC	liquid chromatography
MGR	macrophage galactose receptor
mHL1	major subunit of the mouse hepatic lectin
mHL2	minor subunit of the mouse hepatic lectin
min	minutes
MS	mass spectrometry
MW	molecular weight
NMM	*N*-methyl morpholine
PE	petroleum ether
Py	pyridine
P2	sephadex G-15
PPTS	pyridinium *p*-toluenesulfonate
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
Quant.	quantitative
rHL1	major subunit of the rat hepatic lectin
rHL2/3	two forms of the minor subunit of the rat hepatic lectin
RP	reversed phase
r.t.	room temperature

SAR	structure activity relationship
SP	solid phase
SPR	surface plasmon resonance
TBDPS	*tert*-Butyl diphenylsilyl
TBDPSiCl	*tert*-Butyl diphenylchlorosilane
t-BuOH	*tert*-Butanol
Temp	temperature
TFA	trifuoroacetic acid
THF	Tetrahydrofuran
TLC	thin layer chromatography
p-TsOH	*p*-toluenesulfonic acid

TABLE OF CONTENTS

Introduction

Liver diseases and the asialoglycoprotein receptor.

1.1 Liver diseases and search for a target.

The liver is probably the biochemically most complex organ within the body. It possesses the enzymes and cofactors for an unparalleled number of metabolic reactions.[1] In addition, it is involved in detoxification, storage of various substances (e.g. glucose, vitamin B12 and iron) and plasma protein synthesis. The liver performs several roles in carbohydrate metabolism including gluconeogenesis, glycogenesis and glycogenolysis. It breaks down insulin and other hormones as well as hemoglobin, toxic substances and most drugs. It also performs several roles in lipid metabolism as the synthesis of cholesterol and triglycerides. Among the proteins produced by the liver are coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, and XI, as well as protein C, S and anti thrombin.[2]

The diseases the liver is prone to can be guessed from its biosynthetic and metabolic activities. Not only are there a multitude of diseases directly related to its functions, but also a great number of infections that can alter its capacities to different levels, e.g. different types of hepatitis, viral infections. The hepatitis C virus (HCV) infection is a major health problem affecting an estimated 170 million people worldwide. Persistent infections occur in more than 70% of the people infected, which may be complicated by cirrhosis and/or hepatocellular carcinoma. A highly effective treatment is not yet available.[3] Hepatitis B virus (HBV) infection causes a wide spectrum of acute and chronic liver diseases ranging from latent infection to hepatocellular carcinoma. In the Asia Pacific area more than 10% of the population have chronic hepatitis B. The overall benefit from the most commonly used therapies is limited for most patients.[4,5]

Because of the numerous liver diseases, targeting of drugs to hepatocytes has a great potential. First of all, hepatic targeting will allow a higher and more efficient concentration of drugs in liver. It will also enable therapies with reduced extrahepatic side effects as well as administration of more powerful drugs whose use without being directed to the site of action may be prevented by extrahepatic side effects.

Gene transfer to hepatocytes should be also of great therapeutic interest, since hepatocytes are responsible for the synthesis of a wide variety of proteins. *In vivo* gene transfer would avoid invasive surgery, and problems arising from viral vectors. In addition, it would be important to address the liver for diagnostic purposes.

The Asialoglycoprotein receptor as liver target.

The asialoglycoprotein-receptor (ASGP-R), also called hepatic lectin or galactose receptor, was originally discovered during studies on the metabolism of serum glycoproteins by Morell *et al*.,[6,7] and since then has called the attention of many research groups. It belongs to the superfamily of Ca^{2+}-dependent (C-type) lectins, i.e. mediates specific interaction with carbohydrates in a calcium-dependent manner.[8-10] In fact, it clears desialylated glycoproteins with terminal galactose or *N*-acetylgalactosamine residues from circulation.[6,11]

The hepatic lectin, as is indicated by its name, is highly expressed on the basolateral (blood facing) surface of parenchymal hepatocytes, which contain 100.000-500.000 binding sites per cell. The receptor does occur extrahepatically in the thyroid, in the small and large intestine, the testis and in the proximal tubular epithelial cells of the kidneys. However, in adult rats it was demonstrated that the extrahepatic binding capacity ranges between 1 and 5% of that found in the liver, reflecting a low level of expression.[12,13]

The ASGP-R receptor belongs to the recycling receptors group. The receptor-ligand complexes are endocytosed by clathrin-coated vesicles and once in the acidic environment of the endosomal compartment (pH 5-6) there is a conformational change, causing dissociation of the ligand-receptor system. The receptor is sorted into recycling vesicles that bring it back to the plasma membrane for re-use, while the released ligands take the degradative route (*figure 1*). Like other recycling receptors, the ASGP-R is endocytosed and recycled constitutively, i.e. in the presence or absence of ligands.[14,15]

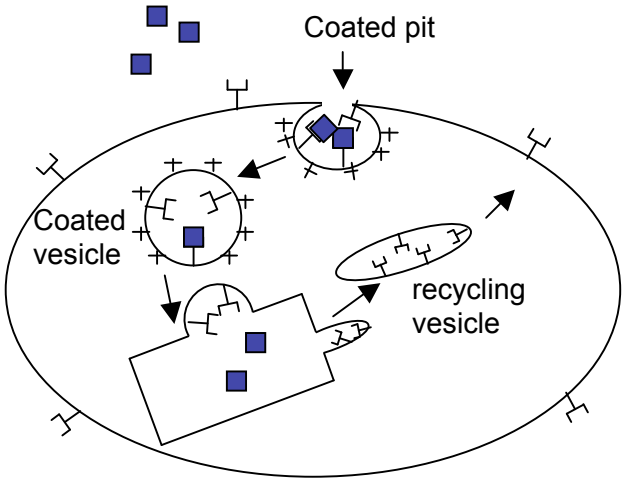


Figure 1: Model for the receptor-mediated endocytosis. Following internalization, the ASGP-R is recycled to the plasma membrane.

Due to the high concentration of the receptor on the liver cells, its specificity, the mechanism of internalization of the complex receptor-ligand, and the high internalization rate, the ASGP-R is a highly interesting target for addressing the liver and a promise for clinical application.

Possible applications of the ASGP-R as a liver target.

Galactosyl-terminated macromolecules can be used as selective hepatotropic vectors of drugs for site-specific drug-delivery. Specific therapeutic agents were successfully targeted to the parenchymal cells of the liver: Biologically active molecules have been covalently linked to galactose-terminated saccharides or incorporated in liposomes,[16,17] as a mean of guiding them effectively to the desired tissues.[7,18]

Conjugated viral nucleoside analogues coupled to galactosyl-terminated macromolecules were used for the treatment of chronic viral hepatitis. The conjugates selectively entered the hepatocytes after interaction with the ASGP-R. The bond between carrier and drug was cleaved in the lysosomes, allowing the latter to be concentrated in the liver. The validity of this chemotherapeutic strategy has

been endorsed by clinical studies.[18] The presence of ASGP-R on the cell plasma membrane in the majority of differentiated hepatocellular carcinoma cells (HCC) and its maintenance on proliferating cells encouraged studies aimed at restricting the action of the inhibitors of DNA synthesis to HCC by their conjugation with galactosyl-terminated carriers and reducing in this way their extrahepatic toxicity.[18]

Interferon (IFN-α) is the most commonly used antiviral reagent in the treatment of hepatitis, but its overall benefit is limited for most patients as there may be insufficient uptake of the cytokine by hepatocytes. Its receptor is present on most cell types in rather low abundance. Directing IFN-α to the ASGP-R by desialylation (asialo- IFN-α) facilitated it's uptake by the liver, and is therefore useful in overcoming the limited antiviral effect of conventional IFN-α.[5]

Attempts of gene transfer and modulation of gene expression to improve or down regulate the biosynthesis of clinically relevant proteins are also under study by addressing the specificity of the ASGP-R. Encapsulation of oligodeoxynucleotides or DNA in liposomes (vehicle able to protect them) labeled with natural ligands of the ASGP-R, resulted in successful internalization.[19,20] In one reported case, transfection was achieved.[20]

It is believed that the hepatic lectin is already the target for hepatitis virions to enter human hepatocytes.[3,21,22] Furthermore, it has been shown to represent a common target for humoral and cellular autoimmune responses in chronic hepatitis, probably contributing to disease perpetuation.[23]

In general, the ASGP-R has been implicated as a site mediating the uptake of viruses as hepatitis A/B and C, leading to diseases that do not have yet an appropriate therapy and can even degenerate to chronic states in some cases. The study of this receptor for the clinic is therefore of interest. Moreover, it allows directing chemotherapeutic agents and foreign genes more efficiently to hepatocytes leading to a localized therapy, which will therefore improve the efficacy and minimize secondary effects.

1.2 Function.

As was mentioned before, the role of the ASGP-R is to eliminate glycoproteins with terminal galactose or *N*-acetyl*g*alactosamine from circulation. However, its true physiological function is still unknown. The existence of a pool of circulating asialoglycoproteins (ASGPs) with potential diagnostic value in patients with liver diseases is not a direct evidence for a regulatory function of serum glycoprotein homeostasis.[6,14] Other proposed functions include the clearance of apoptotic cells and IgA/IgA-antigen complexes, the participation in lipoprotein-(a) *in vivo* catabolism[24], and cell-cell interactions.[8,12]

Despite its at least partially unknown role, its study has provided many insights into the biology and pathology of the liver cells.

1.3 General biological features of the ASGP-R.

The hepatic lectin has been isolated from a number of sources including rabbit,[13] rat,[25] mouse,[26] and human.[27-29] It is a non-covalent hetero oligomer formed by two homologous subunits (H1 and H2 in the human system), which are both required to form high affinity binding sites. However, H1 contains all the necessary signal motifs for endocytosis and recycling with kinetics similar to those of the complete H1-H2 complex.[15] In different species, differences in the number and complexity of the polypeptide subunits that constitute the receptor were found (*table 1* and *table 2*).

Table 1: The two homologous subunits of the ASGP-R.

	Major subunit-1	**Minor subunit-2**
Rabbit	48 KDa	40 KDa
Rat [a)]	rHL1: 41.5 KDa	rHL2/3: 49/54 KDa
Human	H1: 46KDa	H2: 50 KDa

a) rHL2 and rHL3 are encoded by a single gene and differ from each other in the glycosylation pattern but not in the amino acid sequence.

Table 2: Homologies between subunits of the ASGP-R.

Compared sequences	% homology (protein sequence)
H1-H2	58
rHL1-rHL2	51
mHL1-mHL2	49
H1- rHL1	80
H1- mHL1	79
rHL1- mHL1	89

In the case of the human galactose receptor, there is 58% of homology at the amino acid level between the major (H1) and the minor subunit (H2). The interspecies homology is larger for the same type of subunit, being 80% between the major subunits of human and rat, and 89% between mouse and rat. In the case of the minor subunits, the homology is smaller, being 63% between rat and human.[26,29,30]

Each subunit is a type II membrane protein, i.e. it spans the membrane with its amino terminus facing the cytoplasm. They can be subdivided into 4 functional domains; the cytosolic *N*-terminus domain of ~40 amino acid residues, the transmembrane domain and the exoplasmic *C*-terminus of ~230 amino acid residues. This last portion is composed of a stalk segment of ~80 residues and a *C*-terminal carbohydrate recognition domain (CRD) of ~150 amino acids (*figure 2*). Oligomerization occurs via the stalk segment, which forms a coiled-coil structure (*figure 2*). In the oligomeric receptor, high affinity and specificity of ligand binding are the result of multiple interactions of the CRDs with galactoses in the ligand.[10]

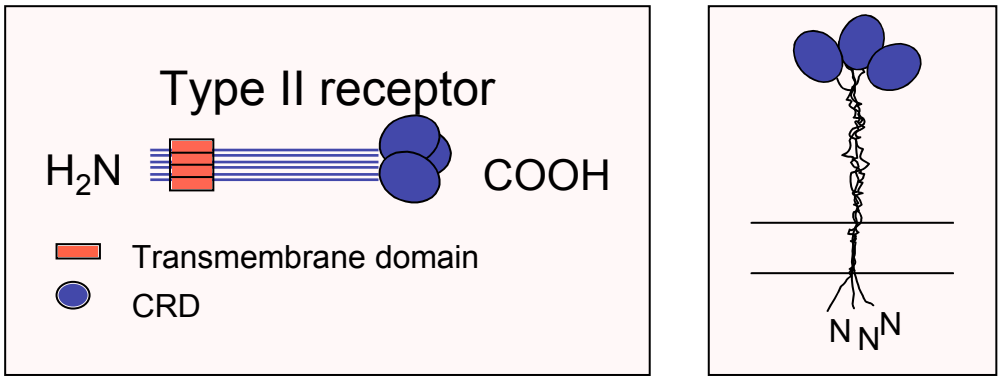


Figure 2: Cartoon representation of the ASGP-R domains and oligomerization via the stalk segment.

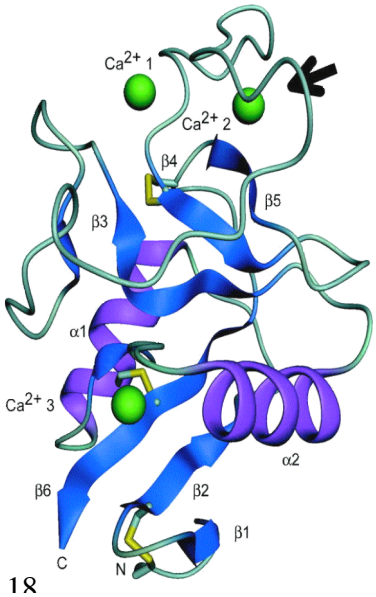
Binding to the ASGP-R.

1.4 The carbohydrate recognition domain (CRD) of H1.

The carbohydrate-binding affinity of C-type lectins is a prerequisite for the receptors to execute their function.

The CRD of subunit H1 of the ASGP-R (amino acid residues 147 to 290) was expressed and co-crystallized with 20 nM lactose in the presence of calcium. The diffraction data were collected by M. Meier *et al.* It is a globular protein with six long and two short β strands and two α-helices. Three disulphide bridges are formed between Cys 153 and 164, Cys 181 and 276 and Cys 254 and 268.[10] (*figure 3*).

Figure 3. From M. Meier *et al.*[10] Ribbon diagram of H1-CRD. α-Helices are shown in magenta, β-strands in blue, calcium ions in green and the three disulphide bridges in yellow. The sugar binds to calcium ion 2 in front of a glycine-rich loop in the upper part of the protein.



The protein contains three calcium-binding sites. Calcium is essential for ligand binding. It is the calcium ion at binding site 2 that is essential for sugar binding. In all three calcium-binding sites of the H1-CRD, the calcium ions are coordinated by eight oxygen atoms. Five of them are arranged into a pentagonal ring, two are located above this plane and one below. At binding site 2, the two ligands above the pentagonal ring are two water molecules that are replaced by the oxygen atoms of the 3- and 4-hydroxyl groups[10,31] of the galactose molecule upon binding. This is reflected by the requirement of these two hydroxyl groups to be free and also their spatial arrangement (equatorial and axial respectively) for the binding to occur. The pentagonal ring in this particular binding site, is formed by oxygens of Gln 239, Asp 241, Glu 252, Asp 265 and Asn 264. Below the plane this Ca^{2+}-ion coordinates with the oxygen of Asp 265 (f*igure 4*).[10]

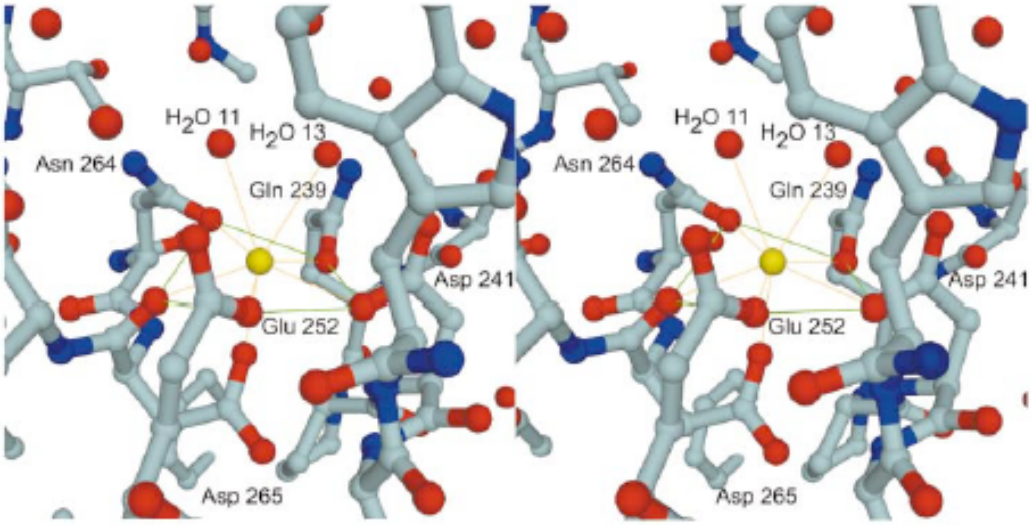


Figure 4. From M. Meier *et al*.[10] Stereo picture of Ca^{2+} binding site 2 of H1-CRD, which is the sugar-binding site. The axial water molecules 11 and 13, are replaced by the 3-hydroxyl and 4-hydroxyl groups of the carbohydrate upon sugar binding.

1.5 Receptor specificity and binding mode.

The mammalian hepatic lectin binds asialoglycoproteins specifically with terminal D-galactose (Gal) or D-*N*-acetylgalactosamine (Gal*N*Ac) moieties. In competitive assays performed with the rHL1, Gal*N*Ac competes approximately 60-fold more

effectively than galactose for binding to the CRD. Interestingly, this preferential binding of Gal*N*Ac to the rat hepatic lectin is not a property of the ASGP-R of rat peritoneal macrophages, even though the CRDs share 85% sequence identity.[32,33] For rabbit ASGP-R the preferential binding is also observed for Gal*N*Ac, being the inhibitory power between 10 to 30-fold better than the one for Gal.[34,35] (*table 3*)

Table 3: Inhibition of ^{125}I-asialo-orosomucoid binding to isolated hepatic lectin and to rabbit hepatocytes by Gal/Gal*N*Ac. Data extracted from Connolly *et al*.[34]

Inhibitors	Isolated lectin (IC_{50} μM)	Hepatocytes (IC_{50} μM)
Asialo-orosomucoid [a)]	0.005	0.003
Gal	1700	1900
Methyl β-D-Gal	1000	1000
Methyl α-D-Gal	1600	1900
Gal*N*Ac	90	70

a) Asialo-orosomucoid is a natural ligand of the ASGP-R.

Beyond the specificity for terminal Gal/Gal*N*Ac, clustering of glycosides enhanced the affinity of certain ligands towards the hepatic lectin. Mono-, bi-, tri- and tetra-antennary oligosaccharides with terminal galactoses bind with increasing affinities to the rabbit hepatic lectin, with dissociation constants of 10^{-3}, 10^{-6}, $5x10^{-9}$ and 10^{-9} M respectively.[34,36] An important difference between the isolated rabbit protein and the hepatic lectin on the cell surface was that the former displays only slightly greater affinity for the cluster glycosides relative to the mono derivatives. This difference may reflect a structural or organizational difference between the isolated and the intact receptor in the membrane.[34]

For the rabbit ASGP-R, it was shown that the anomeric configuration of the glycoside or the substitution of sulfur for oxygen at the anomeric center did not affect binding affinity significantly.[34] However, a bulky aglycon α-anomerically linked to galactose might interfere with the binding.[37]

Similar results from NMR in the case of rHL1-CRD and the crystallographic model of a mutant of the mannose binding protein with the same binding specificity as the

ASGP-R showed that the 6-hydroxyl is directed away from the binding site.[32] This is in agreement with data presented by R.T. Lee *et al*.[37,38] indicating that the binding site for the C-6 region of Gal is rather spacious and capable of accommodating a bulky substituent. Moreover, the environment in this region of the lectin is rather hydrophobic.[38]

There may be a hydrophobic region of rHL1-CRD that can accommodate small alkyl chains of the 2-substituent of galactosamine. Binding of a series of *N*-acyl derivatives of galactosamine to rHL1 suggested that the 2-substituent interacts with the surface of the hepatic receptor with higher affinity when the length of the chain increased from formyl to propionyl. However, more bulky substituents lower the affinity substantially.[32,39]

Molecular modeling performed in our institute[40] confirmed that, as also indicated in the crystal structure of H1-CRD, the 3-OH of the sugar moiety must be equatorial and the 4-OH must be axial for binding to occur. It showed as well the possibility to modify the 2- and probably the 6-positions of the sugar ring. The substituent at the 2-position of Gal is directed towards the core of the protein, and in this region either H-bonds or hydrophobic interactions can be established (Tyr 272, His 256, Asn 264, Asp 266). The 6-position of Gal is pointing towards the surrounding water, but may be extended to reach Trp 243, suggesting a hydrophobic interaction (*figure 5*). These findings are in accordance with the literature as summarized above.

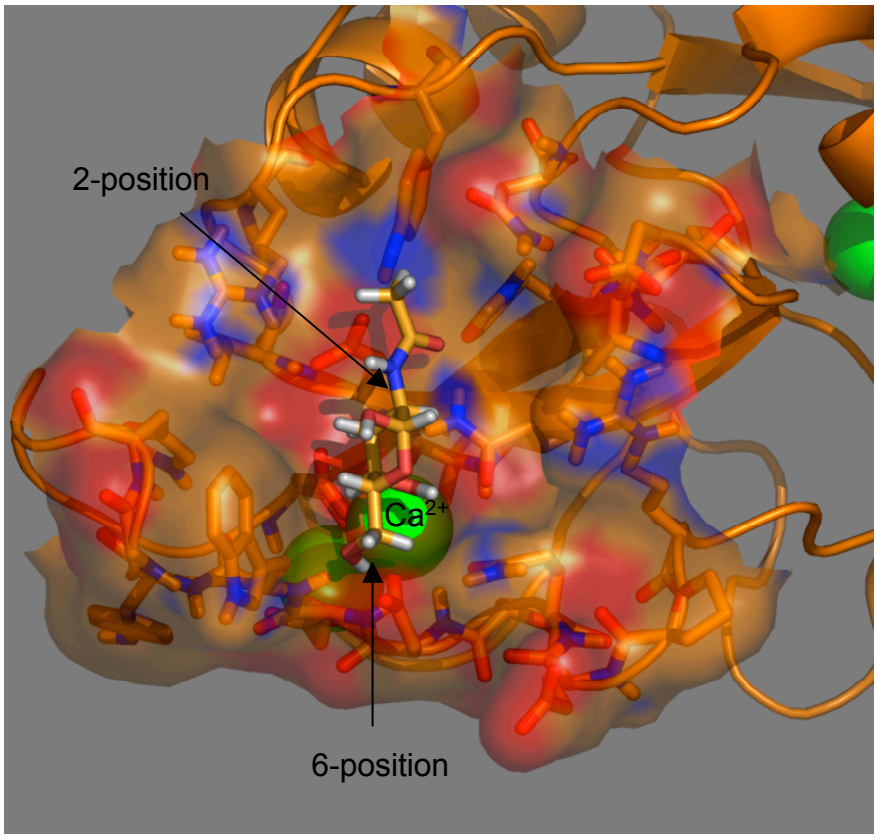


Figure 5: Model of the H1-CRD with Gal*N*Ac bound. Calcium ions are in green, 3- and 4-OHs are coordinated to the Ca^{2+}. The *N*-acetyl group at the 2-position of the sugar is directed towads the core of the receptor and the 6-OH is pointing to the surrounding water. Picture courtesy of Markus Lill, Institute of Molecular Pharmacy, University of Basel.

To summarize the information about the binding mode of a galactose residue to the H1-CRD:

- The 3- and 4-OHs of the galactose moiety should remain unsubstituted for binding to occur.
- The substituent at position 2 of Gal may help to gain affinity by either hydrophobic interactions or hydrogen bonds. However, only a short extension is tolerated.
- The substituent at position 6 of Gal may help to increase the affinity by hydrophobic interactions. Bulky groups are accepted by the receptor.

1.6 Ligands of the ASGP-R reported in the literature.

Synthetic glycosides have become important tools for the elucidation of the binding requirements of the ASGP-R (mentioned in section **2.2**). Numerous research groups investigated the binding properties of the ASGP-R. A literature status is summarized in **annex 1**.

Aim of this work.

1.7 General strategy.

The aim of this work - in the context of an interdisciplinary project on the ASGP-R at the Institute of Molecular Pharmacy, University of Basel (Prof. B. Ernst) - is to improve the understanding of the structural requirements for simple mono- *galacto*-derivatives for improved binding to the H1-CRD. Moreover, it is to provide experimental data of synthetic simple galactose derivatives and mimics thereof for the human H1-CRD. These data can be then used, together with the crystal structure of the H1-CRD, for more accurate *in silico* studies. This approach will lead to novel and improved ligands for this receptor. The work is based on the integration of results from various synthetic efforts and disciplines:

- Synthesis in solution and on solid phase of galactose derivatives and mimics thereof, modified at position 2 of the sugar ring.
- Synthesis of directed libraries of small molecules based on *lyxo*-hexitol derivatives as mimics of galactose, to identify the optimal modification at the 6-position of Gal.
- Solid phase synthesis of a library of mimics of galactosamine derivatives combining modifications at positions 2 and 6 of the *galacto* moiety.
- Determination of the IC_{50} values of members of all three libraries by a competitive target-based assay.
- Ligand ranking and determination of dissociation constants by surface plasmon resonance analysis.

Results of this study are expected to give guidance for the rational design of new and improved ligands for the human hepatic lectin.

The goal of this project is mainly focused in the synthesis of simplified galactose derivatives and mimics thereof as ligands for the H1-CRD. The knowledge base are experimental data available for analogous receptors present in other species, as well as in house molecular modeling data[40] (section **2.2**). For fulfilling our objectives, approaches in solution and on solid phase were established. Five varied small libraries of *galacto*-derivatives were synthesized and analyzed through competitive assays and surface plasmon resonance experiments providing the IC_{50} and kinetic values for synthetic small ligands of the H1-CRD.

1.8 Carbohydrates as ligands.

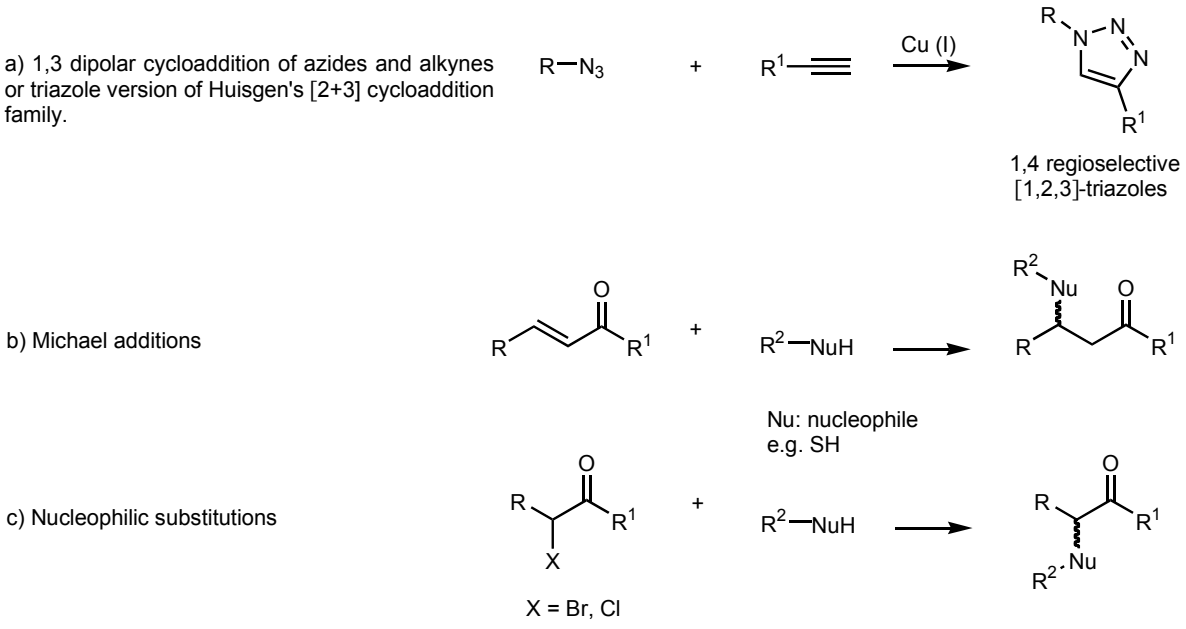
Oligosaccharides play many important roles in biological processes like cell-cell recognition, viral/bacterial-host cell interaction, cellular trafficking, cellular differentiation, etc.[41] It is not surprising therefore, that saccharides are important constituents of drugs (antibiotics, cardiac glycosides, gangliosides).[42] However, most natural carbohydrate ligands bind with low affinity to their receptors (K_D normally in the range of μM to mM), one of various drawbacks for carbohydrates as drugs.[43,44] That is also the case for non-clustered Gal/Gal*N*Ac binding the ASGP-R (*table 3,* section **2.2**). Nature often employs oligovalency to overcome this problem. With multiple carbohydrate-protein interactions the weak monovalent interaction can be amplified. Succesful examples (improvement of affinity by up to a factor of one million) are reported.[45]

A key feature of the binding of an oligosaccharide to the binding site of a receptor is that only a portion of the oligosaccharide is usually in an intimate contact with the protein. Often, as it is the case for the hepatic lectin, only one sugar residue, the so-called “key sugar”, is in contact into the protein. Therefore, molecules containing the key monosaccharide core with modifications unrelated to the sugar, have been synthesized. This strategy is aiming to yield additional interactions with the receptor.[43,44] Such small monosaccharide based inhibitors are attractive lead

compounds since they are of low molecular weigh, hydrolytically more stable and more hydrophobic than natural oligosaccharides.

1.9 Diversity through "click chemistry", Michael additions and nucleophilic substitutions.

A set of powerful, highly reliable and selective reactions for the rapid synthesis of single compounds or combinatorial libraries through a heteroatom link (C-X-C), is called "click chemistry".[46] To be considered as "click", the reaction must be modular, wide in scope and provide high yields. Moreover, the by-products generated by these reactions should be removable by non-chromatographic methods. It should be stereospecific, require only simple reaction conditions. Some examples of this type of reactions include cycloadditions, nucleophilic substitutions and Michael additions (*scheme 1*).



Scheme 1: Introduction of diversity via 1,3-dipolar cycoadditions of azides and alkynes, Michael additions and nucleophilic substitutions.

Huisgen 1,3-dipolar cycloaddition between alkynes and azides are the example "par excellence" for click chemistry. It is a fusion process that combines two unsaturated reactants and provides fast access to an enormous variety of five member

heterocycles. Recently, efforts to control the 1,4- (*anti*) regioselectivity *vs.* 1,5-disubstituted triazoles (*syn*) were successful. Copper-(I) catalyzed reactions, preferentially with the catalyst formed *in situ* yield exclusively 1,4-disubstituted-[1,2,3]-triazoles.[47] (*scheme 1*)

In combinatorial synthesis Michael additions and nucleophilic substitutions on α-halo ketones have often been applied (*scheme 1*). Moreover, a large number of Michael acceptors and α-halo ketones are commercially available ensuring rapid access to diverse libraries.[43,44,48]

1.10 Solid phase synthesis.

The design of small molecules efficiently inhibiting polyvalent carbohydrate-protein interactions is a challenging task for what solid phase chemistry has proven to be a successful strategy.[44] Solid phase synthesis offers the possibility to use an excess of reagents to drive reactions to completion and therefore obtain high chemical yields, and at the same time, eliminating tedious work up and purification steps. In the 1990s, companies applied combinatorial chemistry techniques with the goal to generate large libraries. The actual trend, however, is the tailor-made approach leading to small libraries with high diversity.[49]

One common aspect between solid phase and solution phase synthesis of carbohydrate libraries is the complexity of the monosaccharide building blocks involving multiple similar reactive sites for which the application of a set of orthogonally stable protecting groups is required. However, this small drawback can be minimized on solid phase by using a linker that will act as protective group for some of the reactive positions. The linker has to be stable throughout all other protecting group manipulations and reactions.

In this thesis, SynPhase^{TM} lanterns were used as solid phase support. The core technology is the grafting of a mobile surface polymer onto a rigid unreactive base polymer (*figure 6*). One of the main advantages of the modular nature of lanterns in comparison to resin as a solid support is the ease of handling, avoiding tedious weighing and sometimes inaccurate distribution of the resin into the reaction wells, as

well as minimizing the working volumes. However, the modular nature also implies that for any test to be run on solid phase at least one whole lantern must be loaded, requiring bigger amounts of starting material than the use of resins. The amount of product obtained from a single lantern will provide enough material for biology tests.

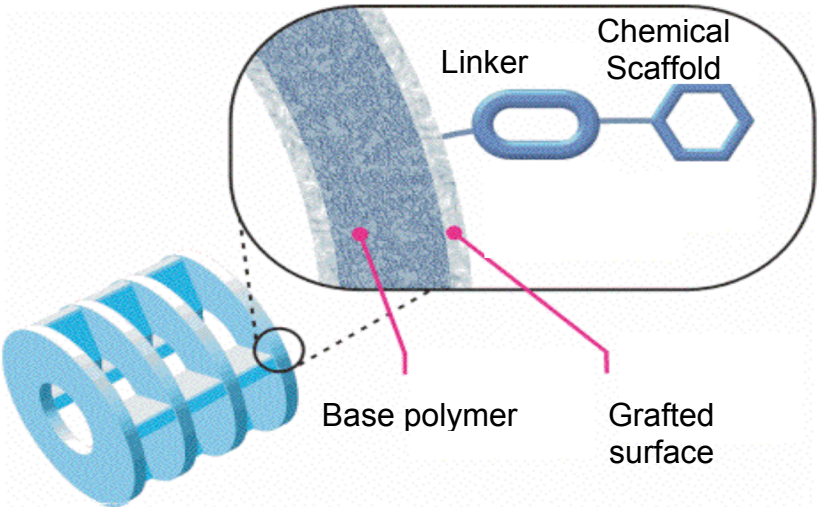


Figure 6. SynPhase^{TM}Lanterns as solid phase support.

1.11 Competitive target-based assay.

To analyze the inhibitory potentials of the compounds from all libraries synthesized, a competitive target-based assay was employed. As target, immobilized H1-CRD expressed in *E.coli* and purified as a single batch, was used.[50-52]

Microtitre plates coated with H1-CRD were incubated with the ligand to be tested in the presence of Ca^{2+} and a competitor complex formed from biotinylated β-Gal*N*Ac-polyacrylamide-polymer and streptavidin peroxidase (*figure 7*). After washing the plates, color development was obtained from the enzymatic reaction between the peroxidase and its substrate (ABTS color development solution consisting of hydrogen peroxide and 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonic acid]). A plate reader at 415 nm then determined the optical density.

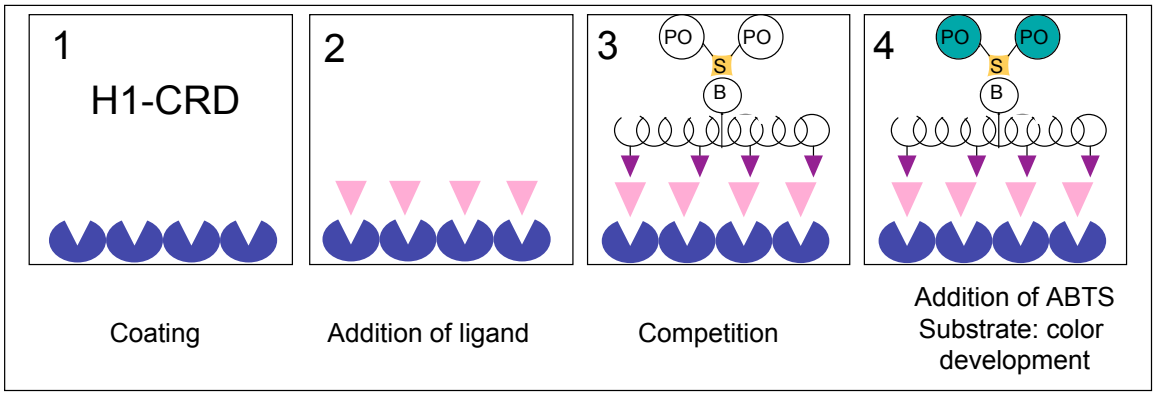


Figure 7. Competitive target-based assay. 1) Plate coated with H1-CRD. 2) The ligand is added to the coated plate. 3) The plate was incubated with the competitive ligand (biotinylated β-Gal*N*Ac-polyacrylamide-polymer-streptavidin peroxidase complex). 4) Addition of peroxidase substrate (ABTS), enzymatic color development. B = biotin, S = Streptavidin, PO = Peroxidase.

The concentrations of ligand required for 50% inhibition (IC_{50}) are determined from inhibition curves using the program Prism4. This method developed by D. Stokmaier[50] proved to be reliable and stable.

1.12 Surface plasmon resonance.

The use of surface plasmon resonance (SPR) technology allows monitoring interactions of macromolecules in real time and label free. Therefore not only specificity can be determined, but also affinity and binding kinetics.

H1-CRD from the same batch used in section **3.5** was immobilized on a sensor chip surface. When ligands are injected in solution, the interaction with the immobilized protein causes a change of mass and refractive index at the gold surface of the sensor chip. These changes can be detected by SPR and plotted as a sensogram. When the solution of ligand is passed over the sensor surface, the sensogram shows an increasing response as molecules interact with the immobilized H1-CRD (association phase). The response remains constant if the interaction reaches equilibrium (steady state). After injection ends, the response decreases as dissociation of protein and ligand occurs (dissociation phase) (*figure 8*).

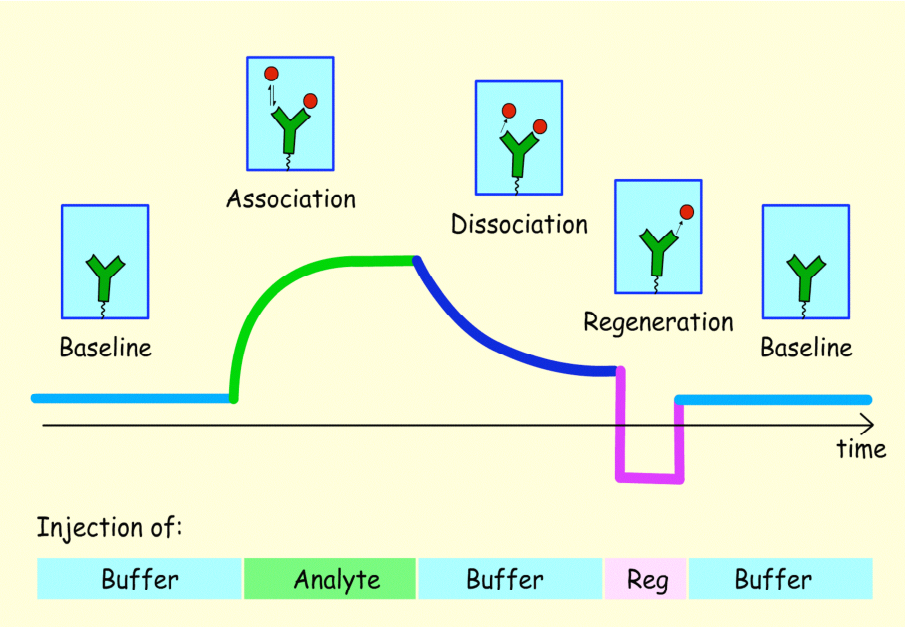


Figure 8. Processes occurring during the different sections of a sensogram from a surface plasmon resonance analysis.

Components of the synthesized libraries were ranked according to their affinities towards H1-CRD. Kinetic values of some of the members were also determined by Dr. D. Ricklin.[53]

Results and Discussion

Linker strategy.

Various small libraries of galactose derivatives and mimics thereof, were synthesized to explore the binding site of H1-CRD and the possibilities of improving the affinity towards the receptor. The ligands were synthesized in solution and on solid phase. Modifications involving only one position of the building block of choice were selected for solution chemistry. In contrast, scaffolds to be simultaneously modified at two different positions were selected for the application of solid phase protocols.

The use of solid phase chemistry introduces two new steps into a library synthesis, loading and cleavage of the products. The linker must allow these two steps to occur in essentially quantitative yields. In addition, it has to be stable throughout all protecting group manipulations and reaction steps.[54] Moreover, it can be seen as a specialized protecting group that can tie up functional groups to reappear at the end of the synthesis. This is very useful in the case of potential ligands for the ASGP-R, in which the 3,4-OHs of the galactose moiety should remain unmodified. As a consequence, the *galacto*-scaffolds, used on solid phase, were covalently attached to the polymer in the form of a 3,4-benzylidene acetal[55-57] (*figure 9*). Acetals are easily hydrolyzed by acids, but they posses virtually unlimited stability to basic conditions. In this way, polymer-bound diol scaffolds can be more easily used in multistep functional transformations.[56,57]

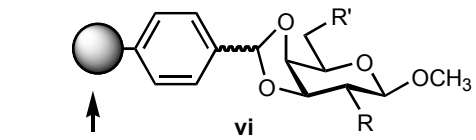


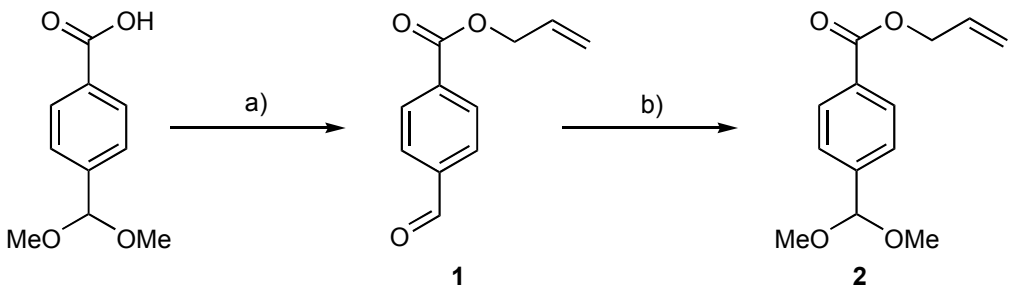
Figure 9: *Galacto*-scaffold attached to solid phase with its 3,4-OHs protected by a benzylidene acetal.

To assure the stability of the linker, it was first tested in solution phase chemistry.

1.13 Synthesis of the linker.

The linker has to be a bi-functional molecule, allowing the attachment to the polymer support and the immobilization of the building block. The attachment to the aminomethylated solid support was established through a stable amide bond formation with a carboxyl containing linker. The immobilization of the scaffold was achieved by the formation of a *p*-benzylidene acetal as discussed above.

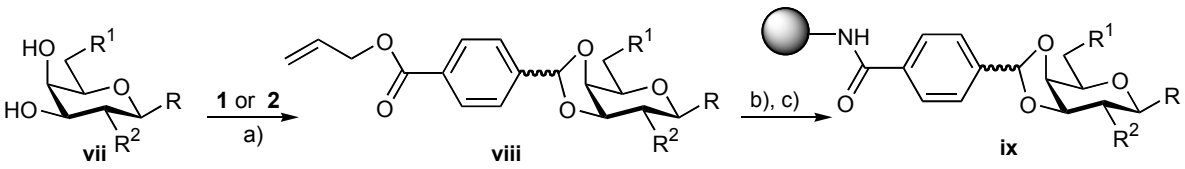
The carboxylic acid function required temporally protection until the loading of the material to the lanterns was performed. The carboxylic acid of 4-dimethoxymethyl-benzoic acid was allyl protected before coupling the linker with the 3,4-OHs of the *galacto*-scaffolds (*scheme 2*). Allyl esters and ethers are often employed as temporary protecting groups. The ease of its formation, its general stability and the many mild methods for its cleavage in the presence of numerous other protecting groups renders the allyl group very attractive.[58,59] Esterification of the carboxylic acid was based on the use of CsF-celite as solid base in acetonitrile.[60] Probably, traces of water present in the base, lead to the unfortunate hydrolysis of the dimethoxyacetal present in the starting material. The re-formation of the acetal was done using trimethylorthoformate in the presence of catalytic amounts of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O).
Either **1** or **2** can be successfully used as linkers. However, compound **2** proved in different attempts to give better yields in the coupling step with different scaffolds in comparison to aldehyde **1**.



Scheme 2: a) CsF-celite, allyl bromide, CH_3CN, 95°C, 20 h (93%);[60] b) $(CH_3O)_3CH$, *p*-TsOH·H_2O, MeOH, r.t., 30 min (97%).[55,57]

1.14 Coupling of linker and scaffolds.

The building blocks were connected by condensation of **1** or **2** with the 3,4-OHs of the *galacto*-moiety by reflux in CH_3CN in the presence of catalytic amounts of *p*-$TsOH \cdot H_2O$. The reactions were carried out in a Soxhlet apparatus with azeotropic removal of water and absorption on 3 Å molecular sieves.[56,57] A diasteromeric mixture of benzylidene acetals was obtained in different yields depending on the scaffold and linker used (*scheme 3*). After allyl deprotection of **iii**, linkage to the solid support was achieved by an amide bond formation.



Scheme 3: a) *p*-$TsOH \cdot H_2O$, molecular sieves 3 Å, CH_3CN, 100°C, 48 h;[56,57] b) Different methods for allyl deprotection employed; c) HOBt, DIC, DMF, r.t., 15 h.

Influence on the binding affinity of modifications to the 6-position of galactose derivatives.

Possible contributions of substituents in the 6-position are not reported. According to molecular modeling, the 6-OH of Gal is directed towards the surrounding water. However, Trp 243 may be reached with an appropiate extension and the affinity improved by hydrophobic interactions.[40]

Modifications at the 6-position of *galacto*-derivative based on the 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol structure (*figure 10*), which binds to the H1-CRD with an affinity comparable to Gal, were introduced. Despite intensive work reported in the literature,[104] little was done with this particular core structure to the best of our knowledge. The lack of the anomeric center renders scaffold **3** highly attractive for a number of reasons. Firstly, the derivatives synthesized will be less prone to metabolic modifications.[45] Secondly, the reduced number of H-bond donors on the new derivatives improves their drug-like character compared to monosaccharide derivatives. Finally, the use of **3** reduces the number of tedious protecting and deprotecting steps.

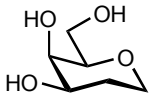


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Figure 10: 1,5-Anhydro-2-deoxy-D-*lyxo*-hexitol

For the introduction of diversity, two families of compounds using Michael additions or nucleophilic substitutions and 1,3-dipolar cycloadditions of azides and alkynes were synthesized.

1.15 Michael additions with OH as nucleophile.

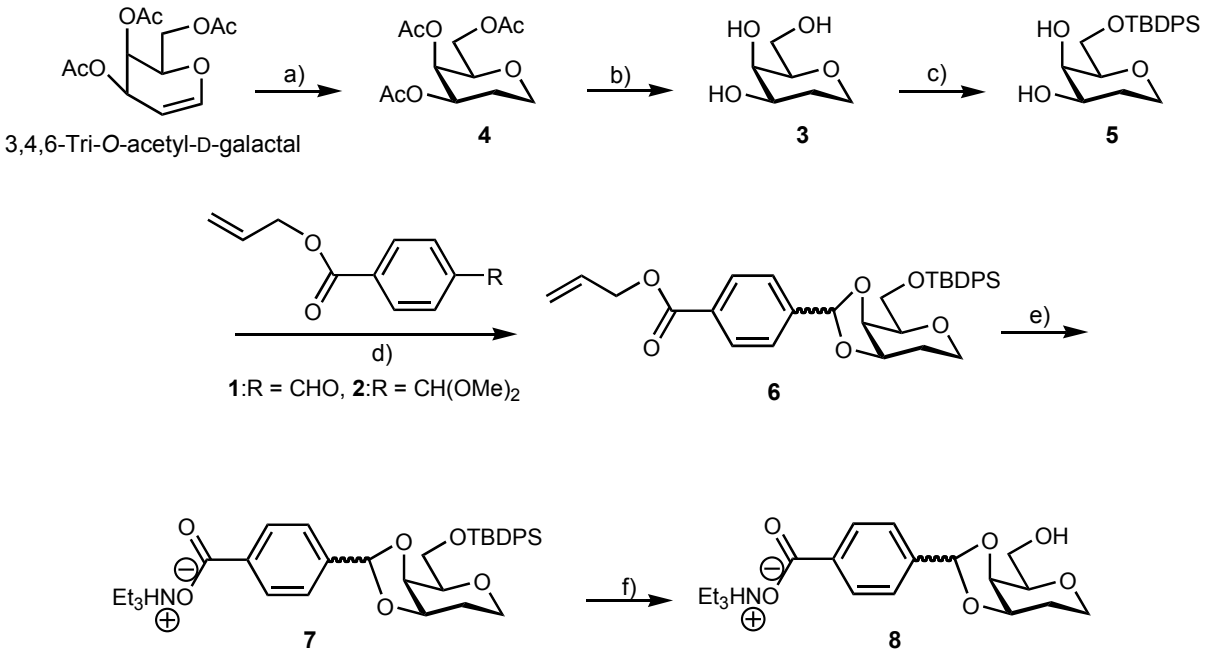
Michael additions, also known as conjugated additions, are powerful transformations that allow the introduction of great diversity due to the numerous commercially available Michael acceptors.[43,44]

Synthesis of the scaffold

The 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol core was synthesized as scaffold for performing Michael additions on its 6-hydroxyl (*scheme 4*). The 3,4-OHs were protected as a benzylidene acetal by the linker **1** or **2**. This strategy also allowed studying the robustness of such a linkage under different synthetic conditions.

3,4,6-Tri-*O*-acetyl-D-galactal was reduced to its 1,5-anhydro-D-*lyxo*-hexitol form. After de-acetylation under Zemplén conditions, *tert*-butyldiphenylsilyl (TBDPS) was selectively introduced at the 6-position. This protecting group was selected for its stability especially to acid hydrolysis[61] (100-250-fold compared to *tert*-butyldimethylsilyl),[59,62] making it ideal for resisting the long acid catalysis at high temperature required for the benzylidene acetal formation[61] between the linker **1** or **2** and compound **5**. The coupling reaction yielding **6** was performed in a Soxhlet apparatus with continuous azeotropic extraction of water. The allyl group was then removed by transition-metal catalyzed isomerization leading to **7** in 88% yield. Tetrakis triphenylphosphine palladium (0) ($Pd(PPh_3)_4$) provided neutral conditions[63] (compatible with the acid labile acetal) for the allyl removal and acetylacetone acted as allyl scavenger.[64] Finally, the free 6-OH required as nucleophile for the conjugated additions, was obtained by selective removal of the silyl ether with tetrabutylammonium fluoride (Bu_4NF). Fluoride ion in an aprotic medium is a powerful agent for the removal of silyl ethers in general, a key discovery done by Corey and Venkateswarlu.[65]

The scaffold **8** was obtained in 6 synthetic steps with 62% of overall yield.

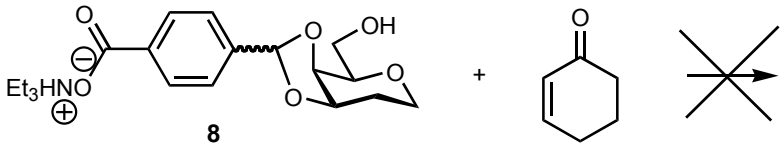


Scheme 4: a) H_2, Pd/C, MeOH, r.t., 15 h (99%);[66] b) NaOMe, MeOH, r.t., 4h (quant.);[66] c) TBDPSiCl, Et_3N, DMAP, DCM, r.t., 24 h (76%);[67] d) *p*TsOH·H_2O, CH_3CN, 100°C, 48 h (94%) when **2** was used, (67%) when **1** was used;[56,57] e) $Pd(PPh_3)_4$, PPh_3, acetylacetone, THF, r.t., 21 h, column chromatography with Et_3N (88%);[64] f) Bu_4NHF, THF, r.t., 22 h (quant.).[65]

Trials for Michael additions

Different conditions for conjugated additions were tried on **8**. Unfortunately, the expected reaction was not observed even after extended reaction times and the employment of several bases (*table 4*).

Table 4: Conditions tested for Michael additions of 6-OH as nucleophile to 2-cyclohexene-1-on as Michael acceptor.



Entry	2-cyclohexene-1-on	Base	Solvent	Temp.	Time
1 [a)]	2.0 eq	33 eq Et_3N[43]	DCM	r.t.-40°C	28 h
2 [b)]	2.0 eq	28 eq Et_3N	DMF	50-90°C	24 h
3 [c)]	2.0 eq	28 eq Bu_4NF[68]	DCM/THF (4:1)	r.t.-50°C	36 h
4	2.0 eq	3.2 eq NaOMe[69]	DCM/MeOH (4:1)	r.t.	72 h
5	2.0 eq	10 eq DBN	DCM	r.t.	120 h

a) r.t. for the initial 9 h, then warmed to 40°C; b) 50°C for the first 8 h, then warmed to 90°C; c) DCM/THF (4:1), warmed only the last 8 h.

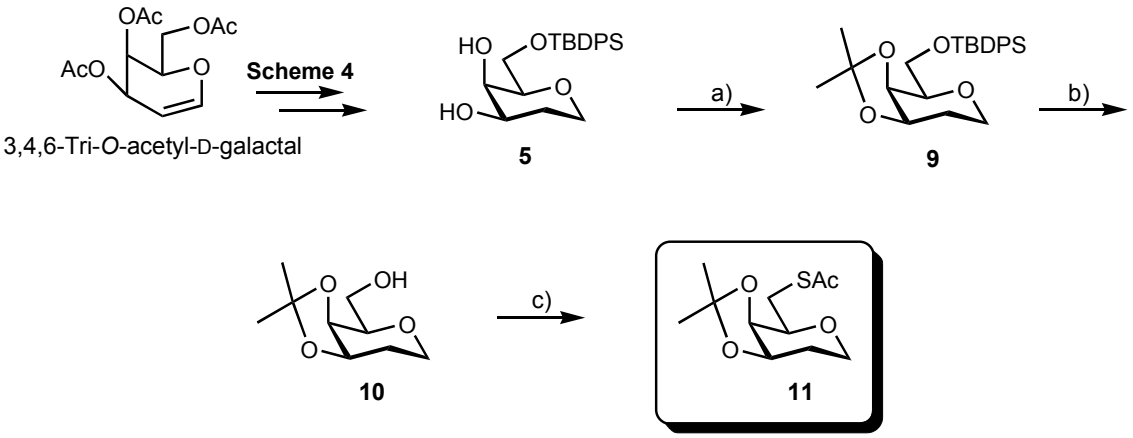
In none of the cases the anticipated product was obtained and **8** could be recovered. The amount of alkoxide present depends on the pK_a of the base. However, increasing the pK_a of the base (Et_3N $pK_a \cong 11$, DBN $pK_a \cong 13$, MeO^- $pK_a \cong 16$) did not result in the expected addition.

1.16 Michael additions or nucleophilic substitutions with SH as nucleophile.

In contrast to the poor nucleophilicity of alcohols, thioles are among the best nucleophiles. To exploit the possibilities of high diversity offered by Michael additions and nucleophilic substitutions on α-haloketones, the 6-hydroxyl was replaced by a thiol group.

Synthesis of the thio-scaffold

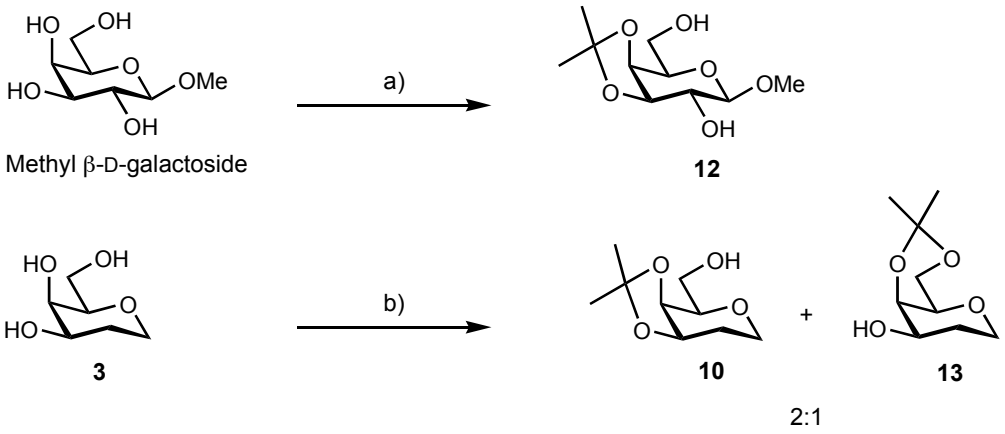
Starting from **5** (*scheme 4*), the 6-*S*-acetyl-1,5-anhydro-2-deoxy-6-thio-D-*lyxo*-hexitol derivative **11** (*scheme 5*) was synthesized and used after *in situ S*-deacetylation as nucleophile in conjugated additions and nucleophilic substitutions to Michael acceptors and α-haloketones. The thio-acetate **11**, was obtained after 6 synthetic steps from D-galactal with 47% of overall yield.



Scheme 5: a) 2,2-dimethoxypropane, CSA, CH_3CN, r.t., 62 h (92%); b) Bu_4NF, THF, r.t., 1 h (97%);[65] c) PPh_3, DEAD, HSAc, THF, -10°C to r.t., 39 h (70%).[70]

The acetonide group was introduced to protect the 3,4-hydroxyls of **5** (92%). This transformation produced an interesting change in the coupling constants of the directly affected protons indicating a conformational change (as an example H-4: **3**→**10**, $J_{3,4}$ = 1.4 Hz→$J_{3,4}$ = 5.6 Hz). The order in which the acetonide and the silyl ether were introduced was relevant. Whereas the protection of the 3,4-OHs by the acetonide in the fully deprotected methyl β-D-galactopyranoside is regioselective, that was not the case for **3** (*scheme 6*). Neither solvent nor temperature changes improved the selectivity (*table 5*), and in all cases a mixture of the 3,4- and 4,6-protected derivatives was obtained. This may reflect an increased reactivity of the 6-hydroxyl in the hexitol derivative **3** relative to the 6-OH of methyl β-D-galactopyranoside. Therefore, the TBDPS protection was introduced first in the fully deprotected compound **3** (76%). The selectivity for primary alcohols in presence of secondary ones is well known.[61,67] The combination of the acetonide and the

TBDPS groups leads to an orthogonal protection. After selective deprotection of the 6-position by means of Bu_4NF (97%), the thioacetate group was introduced using Mitsunobu conditions (70%).[71]



Scheme 6: a) 2,2-dimethoxypropane, *p*-TsOH·H_2O, DMF, 80°C, 4 h (quant.); b) 2,2-dimethoxypropane, *p*-TsOH·H_2O, DMF, 60°C, 18 h (quant., 2:1).[72]

Table 5: Lack of selectivity for the 3,4-*O*-sopropylidene introduction on the hexitol derivative **3**.

Entry	2,2-dimethoxy-propane	Solvent	Temp	Time	Yield	**10:13**
1	1.4 eq	DMF	60°C	18 h	quant.	2:1
2	1.5 eq	CH_3CN	90°C	21 h	quant.	2:1
3 [a)]	1.5 eq	CH_3CN	r.t.	35 h	80%	2:1

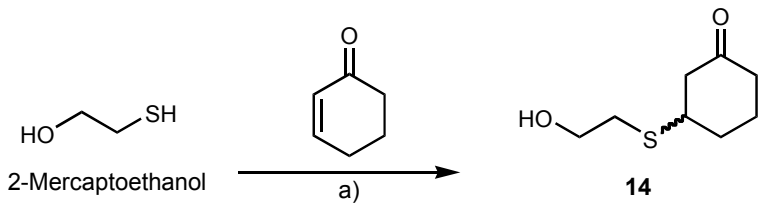
a) The reaction was not complete, starting material was also recovered.

Michael additions and nucleophilic substitutions optimization.

The use of the thio-derivative **11** in Michael additions or nucleophilic substitutions involves a selective removal of the acetyl group to obtain the corresponding free thiol. One-pot *S*-deacetylation and conjugated additions or nucleophilic substitutions procedure according to Bennet *et al*.[69] and modifications thereof[43] were employed.

Diethylamine and piperidine ($pK_a \cong 11$) were tested for the *in situ* deprotection followed by conjugated additions or nucleophilic substitution reactions. However, the

yields obtained were too low (< 39%) for employing the procedure for the generation of a compound library. Lack of good deprotecting capacity of the bases employed was the suspected problem. Two different experiments were run to verify this assumption. On one hand, in a control reaction, the addition of 2-mercaptoethanol to 2-cyclohexene-1-on in the presence of Et_2NH clearly showed that the Michael addition conditions used were adequate to provide the conjugated product in good yields (*scheme 7*). On the other hand, tests were run with **11** in the presence of two different bases (Et_2NH vs. H_2NNH_2·AcOH), but without any Michael acceptor to evaluate the degree of deprotection obtained with each of them (*table 6*).



Scheme 7: a) 2-cyclohexene-1-on, Et_2NH, DCM, r.t., 6 h (72%).[43]

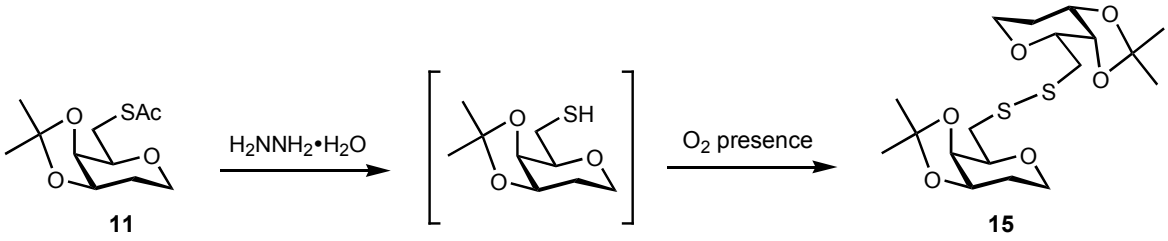
Table 6: Comparative study of thioacetate deprotection procedures in **11**.

Entry	Base	Solvent	Temp.	Time	Deprotection
1 [a)]	Et_2NH	DCM	r.t.	96 h	4%
2	H_2NNH_2·AcOH 6 eq	DMF	r.t.	45 h	quant.

a) 75% of the starting material was recovered

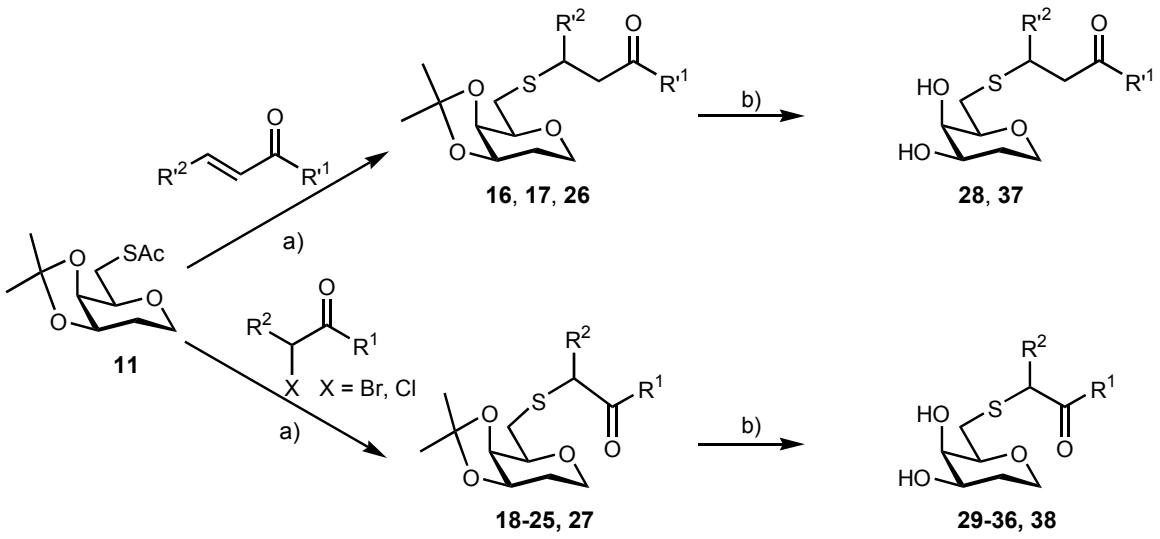
The Et_2NH showed not to be strong enough for deprotecting the thioacetate at the 6-position of the hexitol derivative **11**. Similar problems are reported in the literature.[48] *S*-deacetylation at the anomeric center of Gal derivatives proceeds with excellent yields in the presence of diethylamine.[43] These conditions are however, not sufficient for the same reaction at the 6-position. Due to the proximity of the oxygen atom of the pyranose, a carbonyl group of an anomeric thioacetate is more electropositive and hence increasingly ready to leave.

After deprotection of the thioacetate **11** with the more nucleophilic base hydrazine acetate ($H_2NNH_2 \cdot AcOH$), the Michael additions were still accompanied by a competitive formation of the dimer hexitol-S-S-hexitol-derivative **15** (*scheme 8*). The disulphide formation was suppressed or significantly reduced by working in complete absence of O_2. Solvent, reagents and starting material were degassed and reactions were performed in completely closed and inert systems. The TLCs performed during the experiment, showed that after 4 to 5 h oxidation of **11** was minimal and the freshly added acceptor reacted with the thiol pushing the equilibrium and shortening the time required for quantitative deprotection.



Scheme 8: Oxidation product isolated from reactions without degassing procedures.

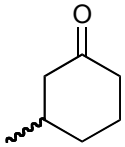
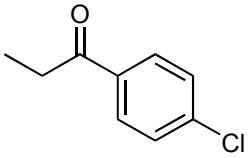
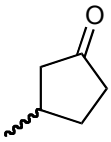
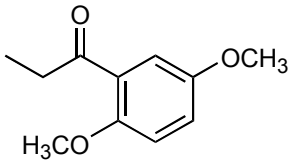
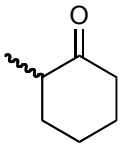
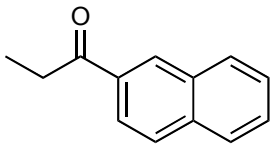
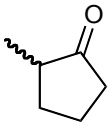
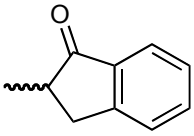
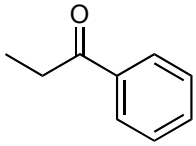
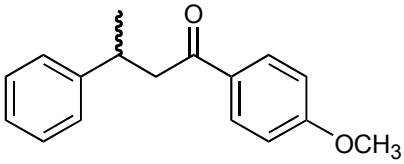
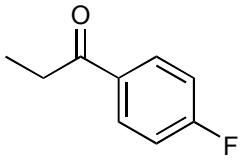
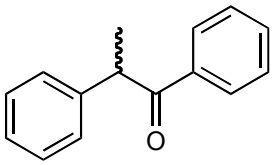
To summarize, the conditions for a one-pot *S*-deacetylation at the 6-position of **11** followed by conjugated additions or nucleophilic substitutions (*scheme 9*), were improved to obtain satisfactory yields. A total of twelve Michael acceptors and α-haloketones were used to sythesize a compound library in solution. Compounds **16-17** and **19-27** were then deprotected according to *scheme 9*. However, due to the delicate manipulations required for avoiding high degree of oxidation even in the presence of small amounts of oxygen, scaffold **11** was not considered ideal for its employment in solid phase chemistry.



Scheme 9: a) H_2NNH_2·AcOH, Et_3N, DMF, r.t., (51-94%);[48] b) aqueous 80% AcOH, 80°C, 3-6 h (60-95%).

Solution library modified by conjugated additions or nucleophilic substitutions.

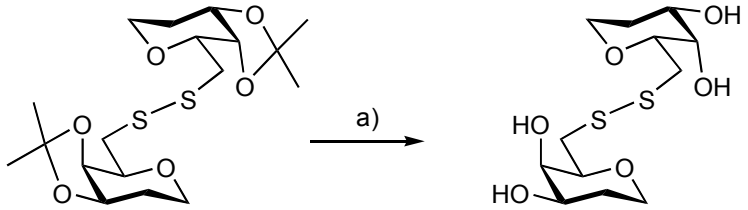
Table 7: Library synthesized by conjugated additions or nucleophilic substitutions. Numeration of 3,4-*O*-isopropylidene-protected derivatives followed by the corresponding deprotected one is shown.

Compound	**R**	Yield[a)]	Compound	**R**	Yield[a)]
16/**28**		51%	**22**/**33**		94%
17 [b)]		14%	**23**/**34**		75%
18/**29**		78%	**24**/**35**		60%
19/**30**		76%	**25**/**36**		65%
20/**31**		77%	**26**/**37**		28%
21/**32**		67%	**27**/**38**		66%

a) Yield of Michael addition or nucleophilic substitution step; b) Because of continuous decomposition, **17** was not deprotected as to form part of the final library.

As expected, no stereoselectivity in the reaction of **11** was observed for the cases of **16** to **19** and **25** to **27**. The diastereomeric mixtures obtained have to be considered as a mixture of substances like any other. As a consequence, results of binding affinity studies obtained with these compounds are to be considered as initial screening experiments. If highly active inhibitors are detected, the problem of the stereoisomers should then be addressed.

The isolated product of oxidation **15** was also deprotected under identical conditions of *scheme 9* to afford compound **39** (*scheme 10*) in 70% yield, that was also submitted for competitive assays.



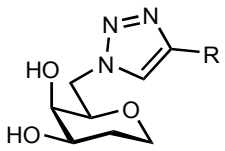
15 **39**

Scheme 10: a) aqueous 80% AcOH, 80°C, 3-6 h, 70%

1.17 Click chemistry at the 6-position of galactose mimics

Copper-(I)-catalyzed [1,2,3]-triazole formation from azides and alkynes, a modified Huisgen 1,3-dipolar cycloaddition, gained high attention in the recent time.[47] On top of the chemical advantages of this reaction,[46] triazoles are very interesting potential pharmacophores.[73]

1,4-disubstituted-[1,2,3]-triazoles were successfully introduced in the 6-position of a hexitol moiety (*figure 11*) by 1,3-dipolar cycloadditions of the 6-azido group of the hexitol and various alkynes. This strategy was also useful for evaluating the potential of this powerful modification for its use in solid phase chemistry.



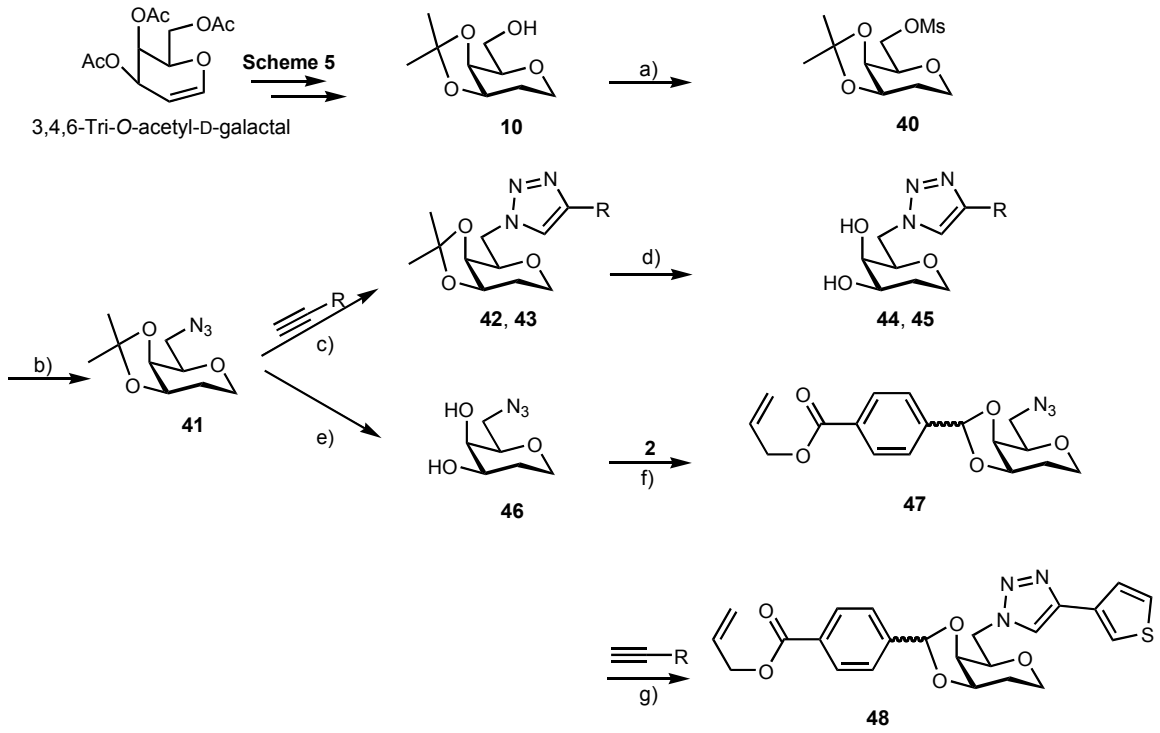
x

Figure 11: 1,4-disubstituted-[1,2,3]-triazoles

Solution phase

To afford 1,4-disubstituted-[1,2,3]-triazoles at the 6-position of a hexitol moiety, an azido functionality had to be introduced. 1,5-Anhydro-6-azo-2-deoxy-D-*lyxo*-hexitol (**41**) was synthesized as scaffold from D-galactal by transforming **10** in the corresponding mesylate (96%), followed by azide substitution (80%) (*scheme 11*). The ^{13}C NMR spectrum showed a characteristic shift in the signal of C-6 (C-6: **3**→**41**: 63.84 ppm→52.04 ppm) indicating the presence of the *N*-linked carbon. Moreover, the finger print region of the IR spectrum of **41** showed the expected very strong signal at 2099 cm^{-1} of the antisymmetric stretching of the azide bonds. The azido-derivative **41** was obtained in 7 steps from galactal with an overall yield of 52%.

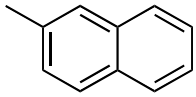
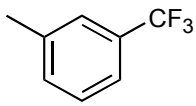
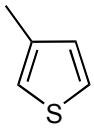
1,4-disubstituted-[1,2,3]-triazoles were regioselectively obtained following the procedure developed by Sharpless and co-workers.[46] The copper-(I) catalyst was prepared *in situ* by reduction of a less costly copper (II) salt ($CuSO_4 \cdot 5H_2O$) using sodium ascorbate as reducing agent. This practice avoided undesired secondary products. In contrast to the standard procedure,[47] several additions of the reagents for *in situ* generation of the catalyst, had to be performed to drive the reactions to completion.



Scheme 11: a) MsCl, Py, 0°C to r.t., 1 h (96%);[74] b) NaN_3,15-C-5, DMF, 100°C, 3.5 days (80%);[74] c) Alkyne, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*-BuOH (1:1), r.t., 2-4 days (67-91%);[47] d) 80% aq AcOH, 85°C, 3 h (95%-quant.); e) 80% aq AcOH, 85°C, 3 h (96%); f) **2**, *p*-TsOH·H_2O, mol. sieves 3 Å, CH_3CN, 105°C, 36 h (44% 1:1);[56,57] g) 3-ethynyl-thiophene, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*-BuOH (1:1), r.t., 40 h (79%).[47]

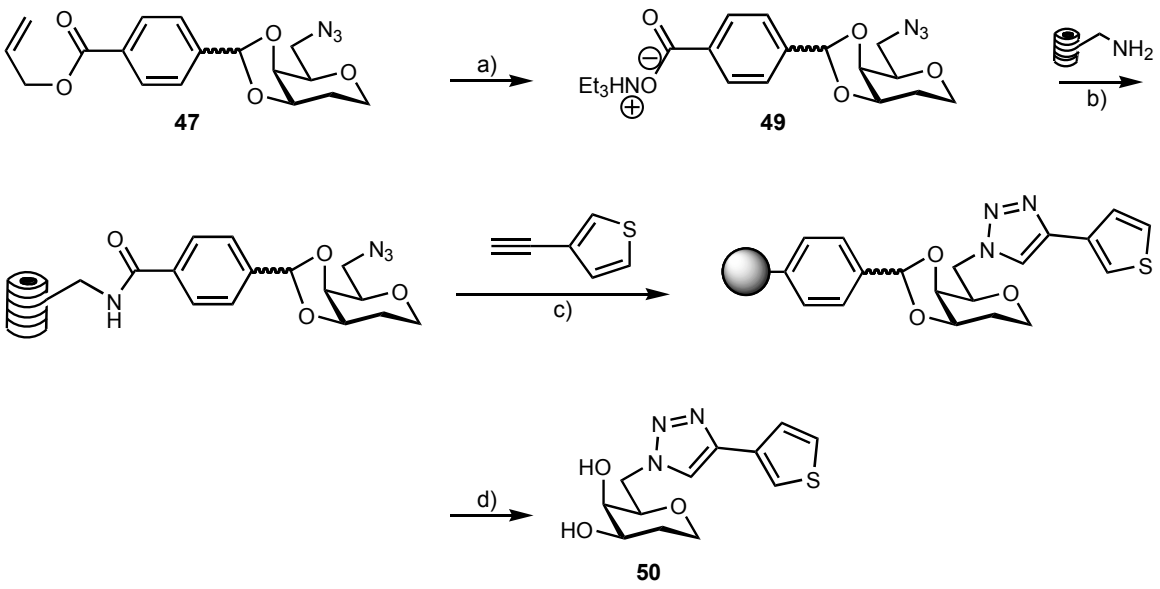
Compounds **42** and **43** were synthesized from **41** as well as compound **48** from the benzylidene acetal-protected compound **47** in good yields in solution (*scheme 11*, *table 8*). The derivatives obtained were subsequently deprotected by acid hydrolysis. After removal of the 3,4-*O*-isopropylidene protection in **41** (96%), an isomeric mixture of 3,4-benzylidene acetals (**47**) was obtained by protection with the linker of choice (**2**) (*scheme 11*). This allowed not only adjusting conditions for future click chemistry on solid phase but also assessing the robustness of the linker under the tested conditions.

Table 8*:* Results of click chemistry step on the hexitol derivatives **41** and **47**.

Protected Compound	**R**	Cycloaddition yield	Deprotected compound
42		91%	**44**
43		67%	**45**
48		79%	**50** (see *scheme 12*)

Trial on solid phase

A test experiment to establish the methodology was carried out (*scheme 12*).



Scheme 12: a) LiOH, H_2O/dioxane (1:1), r.t., 17 h, column chromatography with Et_3N (77%); b) HOBt, DIC, DMF, r.t., 15 h;[75] c) 3-ethynyl-thiophene (3eq x day), sodium ascorbate (0.3 eq x 3/day), $CuSO_4 \cdot 5H_2O$ (0.03 eq x 3/day), H_2O/*t*-BuOH (1:1), r.t., 3 days[47]; d) 80% aq AcOH, 2% TFA, r.t., 2 x 15 h, (58%).[55]

Previous to loading **47** to the activated solid phase, the allyl ester-protection was cleaved under basic conditions with LiOH to afford **49** in 77% yield. The triethylammonium salt of the carboxylate was obtained by performing the purification of **49** in the presence of 0.5-1% of triethylamine. This avoided the acetal cleavage due to acidic silica gel. In addition, the solid support needed to be conditioned. The lantern was first treated with DMF to allow the polymer to swell and then activated with triethylamine, since the aminomethylated lanterns were available in their trifluoroacetate form. Finally, **49** was coupled with the aminomethylated lanterns by using a standard amino acid coupling procedure generating an amide linkage. The click modification was then performed on the immobilized scaffold using a modified procedure (section **5.3.1**). To shift the reaction to completion, excess of reagents and repeated reactions were applied. The product was finally detached from the solid support by acid hydrolysis of the acetal linkage.

50 was obtained in more than 98% purity and 58% of the theoretically loaded material was recovered. The click modification, therefore, proved to be highly valuable for solid phase application.

1.18 Competitive target-based and Biacore assays (In collaboration with D. Stokmaier and Dr. D. Ricklin from the Institute of Molecular Pharmacy, University of Basel).

The competitive target-based assay developed in our institute by D. Stokmaier[50] (section **3.5**) was employed to determine the IC_{50} values of the Gal mimics based on the 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol core.

Surface plasmon resonance technology (SPR/Biacore; carried out by Dr. D. Ricklin[53]) was employed for preliminary monitoring of the interactions between test compounds and the receptor H1-CRD in its monomeric form (section **3.6**).

Results obtained from both methods (competitive assay and Biacore), were carefully analyzed considering the differences and limitations of the asssays. The complementary use of the two assays was extremely helpful for accurate conclusions.

All curves for IC_{50} determination of references and tested samples as well as the protocol employed can be found in **Annex 2** and were kindly provided by D. Stokmaier. SPR fits and methods can be found in **Annex 3** and were kindly provided by Dr. D. Ricklin.

Reference compounds

Galactose, methyl α-D-galactopyranoside, methyl β-D-galactopyranoside, *N*-acetyl-D-galactosamine and synthesized compound **3** served as reference compounds (*table 9*). IC_{50} values were compared with the equilibrium dissociation constants (K_D)

determined by the Biacore assay and both sets of results were validated with data reported in the literature.

All measurements were performed in HEPES buffer containing Ca^{2+} and DMSO. As the IC_{50} values in the absence of DMSO were significantly lower (50%-60%), DMSO was employed independently of the samples solubility.

Table 9: IC_{50} (μM) values determined by competitive target-based assay and equilibrium dissociation constants K_D (μM) determined by SPR for monosaccharides and mimic **3** to be used as reference compounds.

Compound	IC_{50} (μM)	K_D (μM) [b]	Literature
Gal	1754 ± 57	2080	1700 [c]
Methyl α-D-Gal	1280 ± 49	2840	1600 [c]
Methyl β-D-Gal	545 ± 39	1780	1000 [c]
Gal*N*Ac	78 ± 5	112	90 [c]
3	1399 ± 368 [a]	1480	n.d.

IC_{50} values are the media ± standard deviation of two independent determinations except for a) in which three independent determinations were done. b) Obtained from randomized triplicate injections of 8 concentrations between 6.0 mM and 2.7 μM. c) Data extracted from Connolly *et al.*[34] IC_{50} values for isolated hepatic rabbit lectin determined by competitive assay with ^{125}I-asialoorosmucoid. n.d. means “not determined”.

All values obtained for monosaccharide moieties in SPR experiments and with the competitive target-based assay were in the same range. Differences in the absolute values (usually within a factor of 2-3) are the result of the intrinsic difference between the assay types. The IC_{50} values from our competitive target-based assay and the literature ones were in a close range while direct binding values from Biacore were always slightly higher.

In competitive assays, galactose showed the lowest affinity towards H1-CRD followed by methyl α- and β-D-galactopyranoside. However, in the case of SPR, methyl α-D- galactopyranoside showed the lowest affinity. As expected, Gal*N*Ac was

the best binder in both assays with approximately a 20-fold enhancement of the binding affinity relative to galactose.

Literature data for α/β-*galacto* isomers,[34] did not clearly document the preference of the receptor. However, in our competitive assay and the SPR experiment methyl β-D-galactopyranoside was superior by a factor of 1.6 to 2. The Biacore curves obtained with both galactosides clearly show a parallel shift between them. In this case, a direct comparison can be done due to identical molecular weights (*figure 12*).

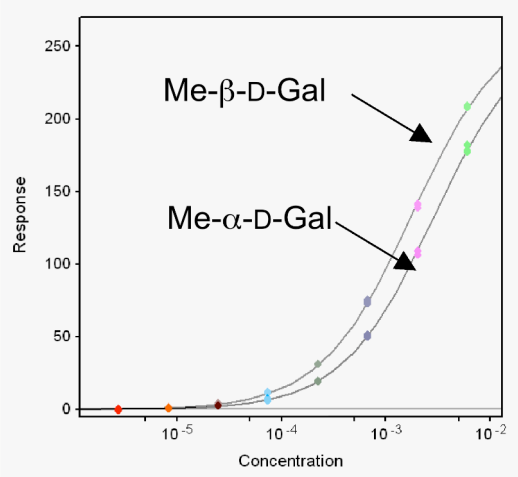


Figure 12: Superimposed Biacore curves of methyl β-D-galactopyranoside (left curve) and methyl α-D-galactopyranoside (right curve). Shows that from both monosaccharides compared, methyl β-D-galactopyranoside is the best binder.

Biacore preliminary ranking.

Hexitol derivatives modified by conjugated additions or nucleophilic substitutions, were submitted for SPR preliminary ranking. It is important to stress the preliminary character of the results obtained by this experiment. Only a single injection at a constant concentration (1 mM) was performed for each compound. Therefore, values from SPR cannot be directly compared. This can be exemplified by the comparison of the results obtained for Gal and Gal*N*Ac in the ranking (*figure 13*) where the 20-fold higher affinity of Gal*N*Ac is not reflected by the SPR signal. The raking experiment serves to detect unexpected or unusual behaviors, such as non-specific binding, and to identify binders with extremely high or low affinities.

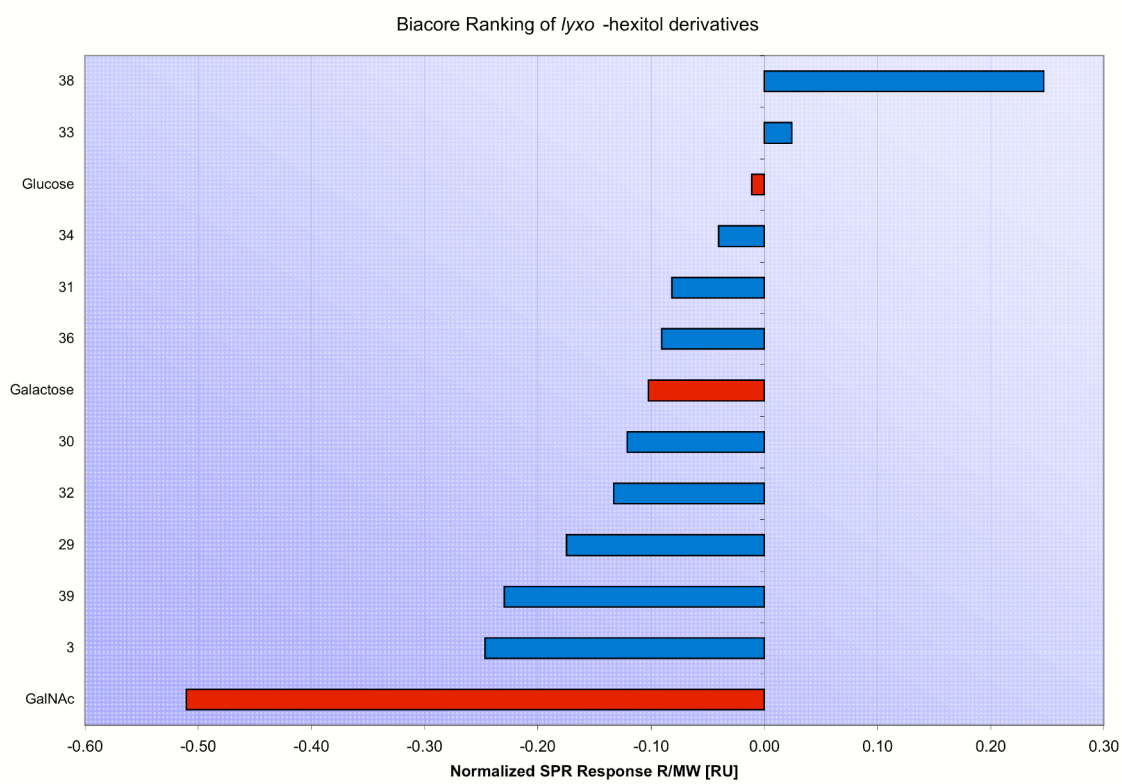


Figure 13: Biacore ranking of the binding affinities towards H1-CRD of the hexitol derivatives modified by nucleophilic substitutions at the 6-position and reference compounds. The data was obtained for immobilized H1-CRD (aprox 8000 RU density equivalent to aprox 8 ng/flow cell) by performing a single injection at a concentration of 1 mM per sample and was then normalized with the corresponding analytes MW.

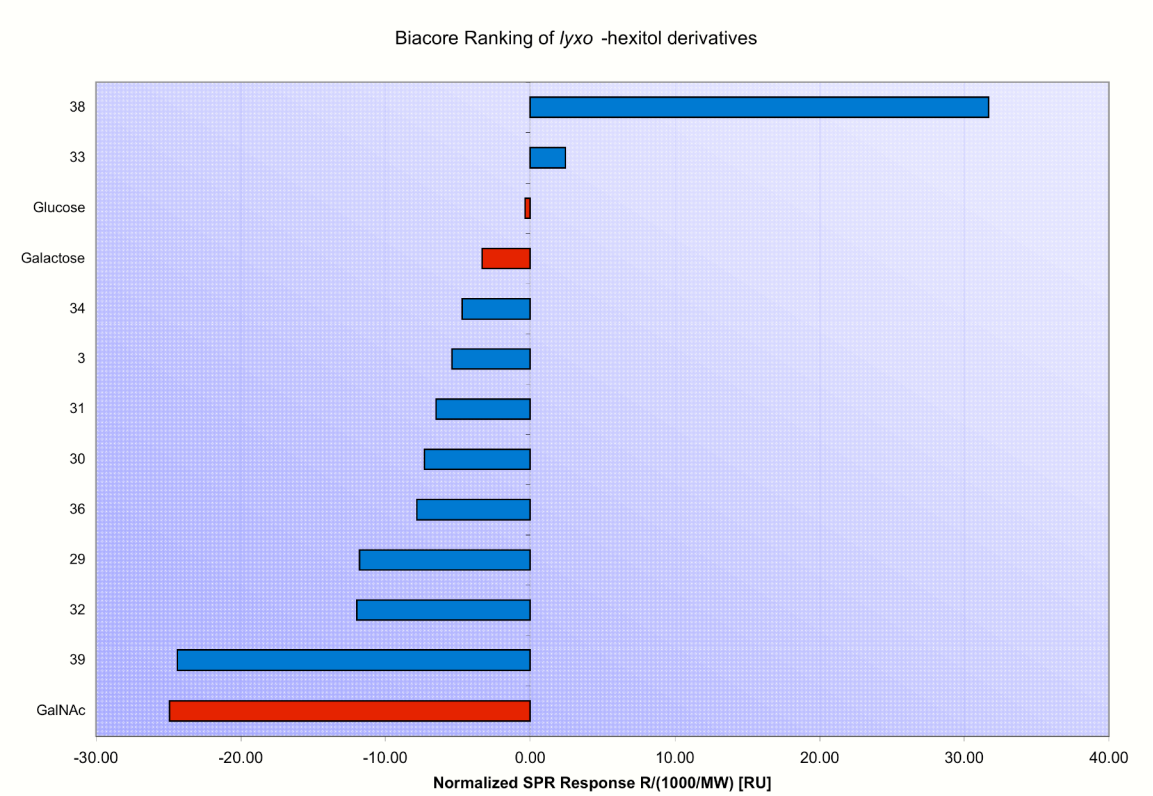


Figure 14: Biacore ranking of the binding affinities towards H1-CRD of the hexitol derivatives modified by nucleophilic substitutions at the 6-position and references compounds. Same original data from *figure 13* was normalized with the reciprocal of the MW (1/MW) of the corresponding analytes.

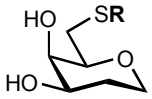
When the MW-normalized data set is considered, only three samples resulted to be worse binders than galactose while the rest showed higher affinity. In contrast, if the reciprocal MW-normalized data set (*figure 14*) is taken into account, all library members tested seem to have improved affinities for H1-CRD compared to galactose. The best binder was compound **39**, the only analyte with a different general pattern. In addition, compound **38** generated a positive signal opposing to the general behavior of the other members and the references used (Gal and Gal*N*Ac) that gave negative signals. Further investigations were therefore performed on **38** and **39** and are discussed in section **5.4.4**.

Competitive target-based assay

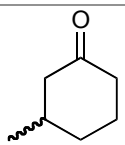
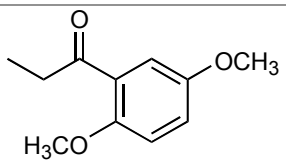
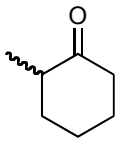
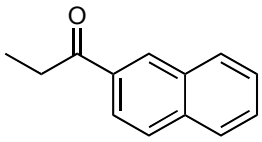
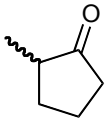
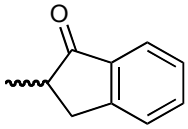
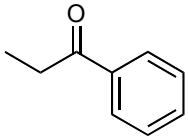
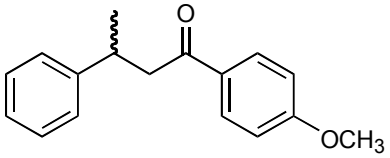
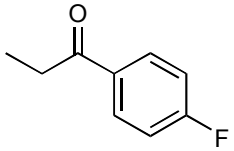
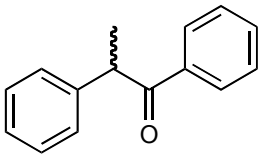
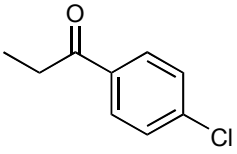
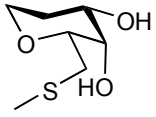
IC_{50} values of hexitols based on nucleophile **11** or [1,2,3]-triazoles derivatives are presented in *table 10* and *11,* respectively. Unless specified, the galactose mimics were tested in replicates with good reproducibility.

In general, the binding affinities towards H1-CRD were approximately 3 times better than those of Gal or compound **3**.

Table 10: IC_{50} (μM) values determined for Gal mimics modified at the 6-position by conjugated additions or nucleophilic substitutions.

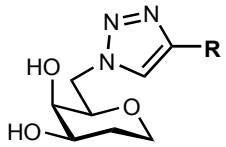


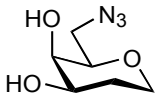
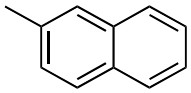
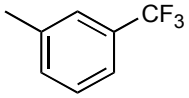
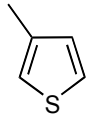
28 to **39**

Hexitol	**R**	IC_{50} (μM)	Hexitol	**R**	IC_{50} (μM)
28		868 [a]	**34**		983 [a]
29		575 ± 15	**35**		n.s.
30		450 ± 3	**36**		346 ± 37
31		519 ± 147	**37**		n.s.
32		472 ± 216	**38**		n.s.
33		524 ± 134	**39**		94 ± 18

a) One determination done. n.s. means “non soluble” compounds in HEPES buffer (even with 5% DMSO). For all other compounds two independent determinations were performed and are informed as the media ± standard deviation.

Table 11: IC_{50} (μM) values determined for Gal mimics modified at the 6-position by 1,3-dipolar cycloadditions.



Compound	R	IC_{50} (μM)
46 [a] 	–	248 ± 41 (K_D = 855) [b]
44		Precipitation[c]
45		481 ± 127
50		421

a) Compound measured was 1,5-Anhydro-6-azido-2-deoxy-D-*lyxo*-hexitol, precursor of the other derivatives tested. b) K_D value also determined with Biacore c) Precipitated during assay. Values are the media ± standard deviation of two independent determinations, except for compound **50**, for which only one measurement was performed.

To summarize, the three kinds of chemical modifications that were carried on the hexitol moiety generated a group of inhibitors that had approximately 3 times enhanced affinity for H1-CRD relative to Gal. However, the binding is still weak and in the order of methyl β-D-galactopyranoside with the exception of the azido derivative **46**. Nevertheless, these findings encouraged future studies with additional modifications in the 2-position of galactose.

Biacore ranking vs. IC_{50} determination and further investigations.

The preliminary Biacore ranking of the hexitol derivatives tested in this chapter predicted a general improvement in the H1-CRD binding affinity relative to galactose. Such behavior was confirmed by the competitive assay.

An interesting finding in both experiments (Biacore and competitive assay) was that compound **39** resulted to be the best binder. In further SPR-experiments, randomized triplicate injections of 8 concentrations (three-fold serial dilutions) between 6.0 mM and 2.7 μM were performed. Negative signals that could be mirrored were obtained showing fast binding kinetics. As expected, the equilibrium responses could be fitted to a simple 1:1 binding model. Nevertheless, a slight signal increase could be observed during steady state (*figure 15*). This is indicative either of a low degree of non-specific binding or a more complex binding mode. However, these deviations were rather small and are not expected to largely influence the binding data. Due to the special characteristics of **39**, which possess two pairs of 3,4-OHs free, it might improve the local concentration. The K_D of 1.13 mM for **39**, was not in the range of Gal*N*Ac as determined in the competitive assay.

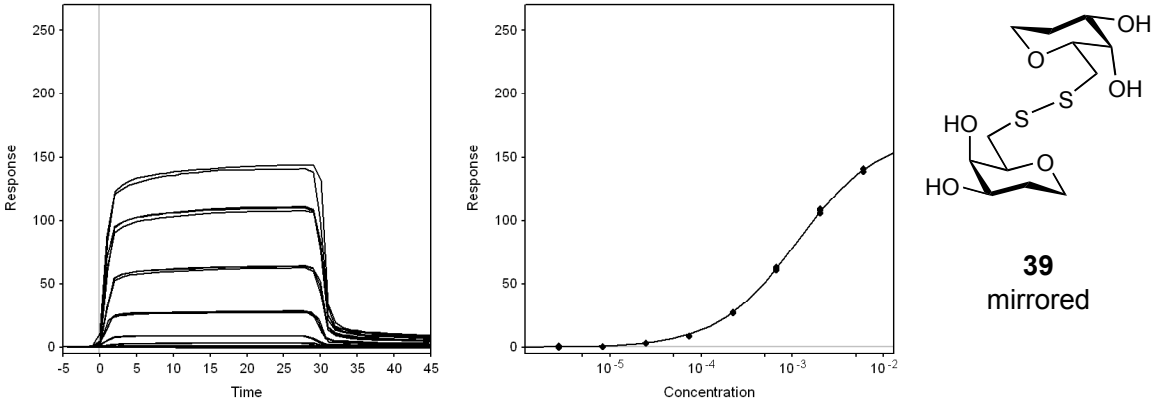


Figure 15: SPR curve obtained for **39**.

In the Biacore ranking, compound **38** appeared to be a good binder but contrary to all other compounds tested the signal generated was positive. As no additional information could be obtained from the competitive assay due to poor compound solubility (precipitated in the buffer during the assay), further investigations were

performed with Biacore. A positive signal with fast kinetics was recorded but no saturation was achieved. Based on a linear relationship between response and concentration, the compound was eliminated from the data set under evaluation. The non-specific type of binding detected can be explained by two possible theories. On one hand, the high hydrophobicity of **38** can generate non-specific hydrophobic interactions. On the other hand, this effect can be also observed if precipitation occurs, as it was the case during the competitive assay.

Influence on the binding affinity of modifications to the 2-position of galactose.

Substituents linked to the 2-position of Gal derivatives have a privileged situation upon binding to the H1-CRD. They are directed towards the core of the protein and can establish either hydrophobic interactions or hydrogen bonds with the various amino acids in this area of the protein (Tyr 272, His 256, Asn 264, Asp 266)[10,32,35,39,40]. Furthermore, Gal*N*Ac competes approximately 10 to 60-fold (depending on the animal species) more effectively than galactose for binding to the H1-CRD.[32,34]

The aim of this particular work was to study the influence on the binding affinity to H1-CRD, of a collection of substituents at the 2-position of Gal with varied characteristics and sizes. Due to possible steric clashes, the length of the chain or size of the substituent may be a limitation.[32,40]

Modifications at the 2-position of methyl α-D-galactosides and derivartives thereof were performed. The compound library was synthesized in solution and on solid phase by two main approaches. On one hand, 1,3-dipolar cycloadditions were performed using alkynes and an azido groups. The later was introduced with a two-carbon spacer attached to the 2-position of Gal. On the other hand, solid phase amino acid coupling was performed with a galactosamine derivative.

1.19 Library in solution

A library of methyl α-D-galactosides in which diversity was introduced by copper-(I)-catalyzed 1,3-dipolar cycloadditions of an azide and alkynes was synthesized in solution. Once again the broad spectrum of advantages offered by click chemistry[46] (reactions discussed in section **5.3**) were put into practice.

When this work was initiated, experimental data were only available for non-human receptors. IC_{50} values for methyl β- and methyl α-D-galactopyranoside from Connolly *et al*.[34] for isolated rabbit hepatocytes are 1.0 mM and 1.6 mM, respectively. Since it

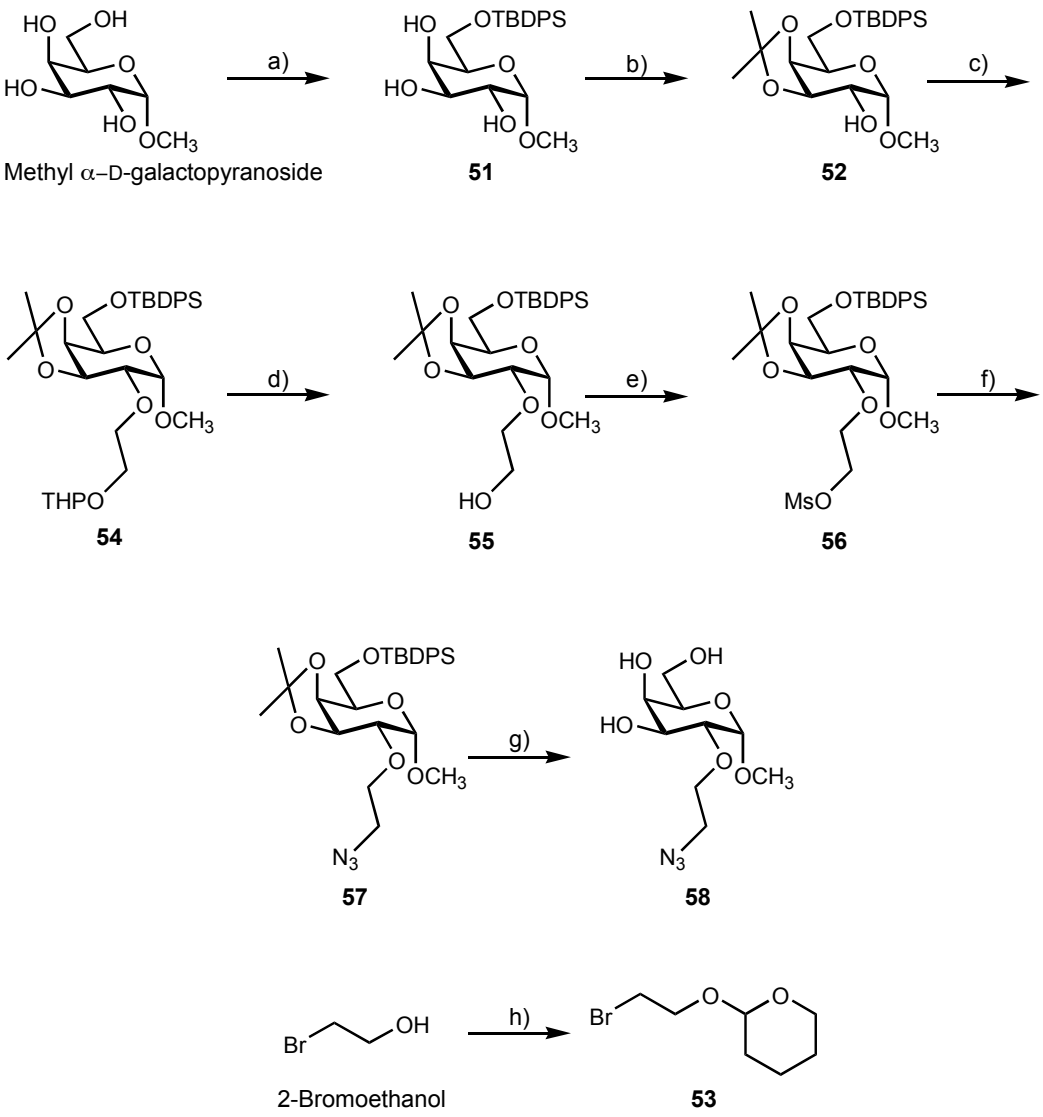
was reported that the anomeric configuration of glycosides or even the substitution of sulphur for oxygen at the anomeric center did not affect binding significantly,[34] the above difference was considered to be an experimental error. Furthermore, there are a number of examples in the literature (see **annex 1**) where the α derivative shows a higher affinity than its β counterpart.[34,35,39] Based on that and due to the ready availability of the starting material, a library of methyl α-D-galactosides was our option of choice. However, in the meantime are found in competitive and in Biacore experiments that methyl β-D-galactopyranoside shows an affinity for H1-CRD two times better than its α counterpart (*table 9*).

Synthesis of the scaffold

Methyl 2-*O*-(2-azidoethyl)-α-D-galactopyranoside (**58**) was synthesized as scaffold of the triazole library. According to *in silico* data,[40] short aliphatic chains at the 2-position of galactose derivatives may generate hydrophobic interactions with the protein. As a consequence, the azido group was connected to the 2-position by a two carbon chain (*scheme 13*).

The desired scaffold **58** was synthesized from the commercially available methyl α-D-galactopyranoside applying orthogonal protection. The synthetic pathway was based on the knowledge of the different order of reactivity of the hydroxyl groups in galactose. The primary hydroxyl is much more reactive than the secondary hydroxyls, the 3-OH is more reactive than the 2-OH, while the 4-OH is the least reactive one. Therefore, the TBDPS group was quantitatively introduced at the primary alcohol.[61] Secondly, the 3,4-diol was protected by 3,4-*O*-isopropylidenation (89%).[58] Thirdly, a two-carbon chain was introduced by alkylation of the 2-hydroxy group of **52** with **53** (50%). During the selective cleavage of THP[58] in the presence of the acetonide[79,80] group only a very low concentration of PPTS was applied. Finally, **55** was transformed in the corresponding mesylate in pyridine (92%) followed by azide substitution (quant.) to obtain the protected azido-scaffold **57**. The selective removal of the TBDPS group with tetrabutylammonium fluoride[65] (quant.) followed by acetonide deprotection by acid catalyzed hydrolysis (quant.) yielded the target

derivative **60**. It was obtained from methyl α-D-galactopyranoside in 7 synthetic steps with an overall yield of 34% (*scheme 13*).

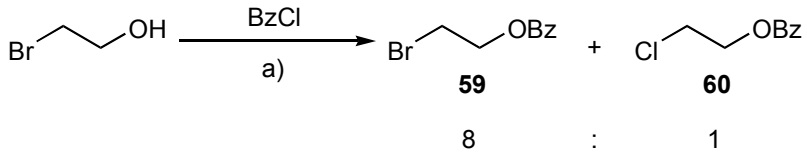


Scheme 13: a) TBDPSiCl, Et_3N, DMAP, DCM, r.t., 64 h (quant);[67] b) 2,2-dimethoxypropane, *p*-TsOH·H_2O, CH_3CN, r.t., 29 h (89%);[72] c) **53**, NaH, anh DMF, 0°C-40°C, 29 h (50%);[81] d) PPTS, MeOH, 55°C, 5 h (83%);[79] e) MsCl, Py, 0°C-r.t., 3 h (92%); f) NaN_3, DMF, r.t.-50°C, 18 h (quant.); g) i) Bu_4NF, THF, r.t., 2 h, (quant),[65] ii) 80% aq AcOH, 50°C, 12 h (quant) h) DHP, PPTS, DCM, r.t., 2 h, (quant).[79,82]

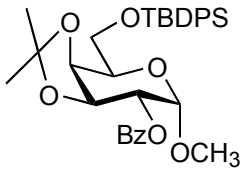
In the synthetic pathway to **58** there were two critical steps. The first one was the careful selection required for the protecting group for 2-bromoethanol. Originally, the basic labile benzoate group was chosen. On one hand, Br/Cl exchange occurred between 2-bromoethanol and benzoylchloride generating a mixture of the expected

3-bromoethylbenzoate (**59**) and the less reactive chloro counterpart **60**. This could be mostly solved by a reduced reaction time (*scheme 14*). On the other hand, a transesterification occurred when trying to introduce the two-carbon spacer to the galacto-derivative. The 2-*O*-benzoyl derivative **61** was instead obtained (*figure 16*). The protecting group of second choice was the also basic labile but more stable pivaloate group to possibly avoid transesterification. Unfortunately the introduction of this group could not be achieved at different temperatures and in the absence or presence of DMAP as catalyst. Finally, THP was employed successfully.

The second critical step was the low yield obtained for the introduction of the properly protected 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran to the 2-position of **52**. The cause was the partial deprotection of the TBDPS group.[58]



Scheme 14: BzCl, Py, 0°C-r.t., 4 h (51%).



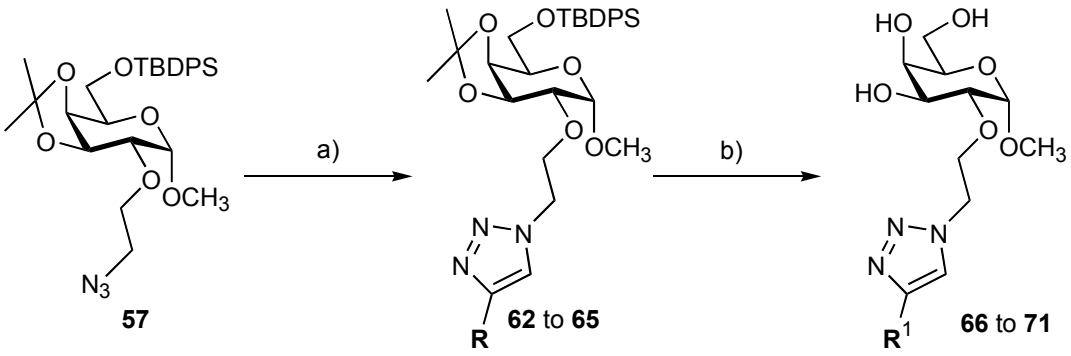
61

Figure 16: transesterification product.

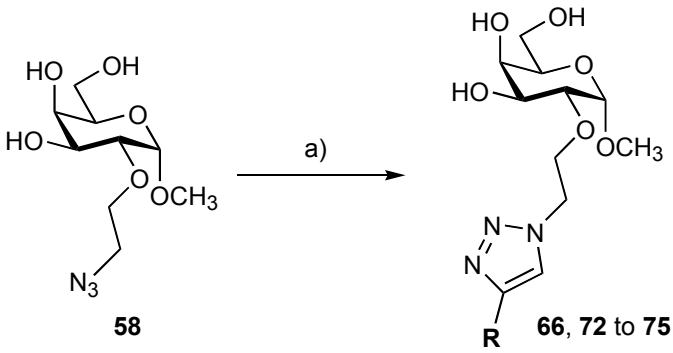
Synthesis of the library.

A collection of nine methyl α-D-galactosides and two related secondary products, modified at the 2-position, was obtained by click chemistry performed in H_2O/*t*-BuOH (1:1). Derivatives were obtained from scaffold **57** (*scheme 15, table 12*). Because the triazol formation from acetylenes and azides is possible in presence of free OH- and NH_2- groups, the reaction is carried out as well with the deprotected precursor **58** (*scheme 16*, *table 13*). In both cases, the standard procedure of Sharpless and co-

workers[47] had to be slightly modified. As in **section 5.3.1**, several additions of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate had to be performed to drive the reactions to completion.

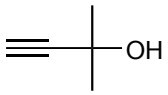
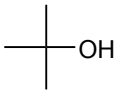
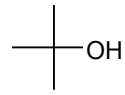
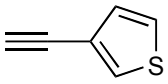
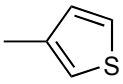
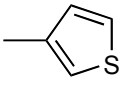
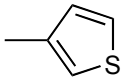
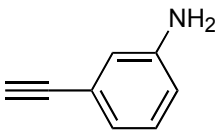
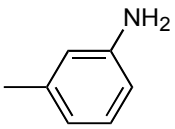
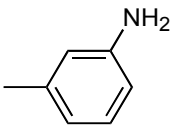
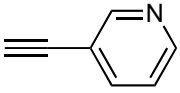
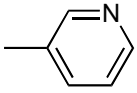
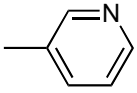


Scheme 15: a) Alkyne, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*-BuOH (1:1), r.t., 1-3 days, (81%-98%);[47] b) i) Bu_4NF, THF, r.t.; ii) 80% aqueous AcOH, 80°C (12%-60%).



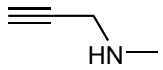
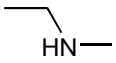
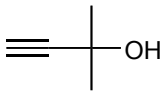
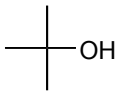
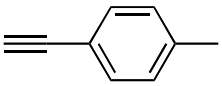
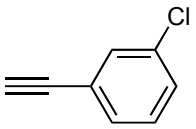
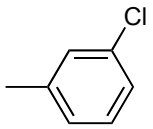
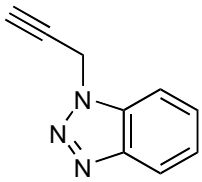
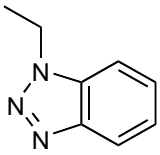
Scheme 16: a) Alkyne, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*-BuOH (1:1), r.t., 1-4 days, (51%-87%).[47]

Table 12: Methyl α-D-galactosides modified at the 2-position by click chemistry, synthesized from the protected scaffold **57**. Products and yields of cycloaddition step and after deprotection are presented.

Alkyne	Yield [a]	R	R^1
	81%	 **62**	 **66** (12%) +  **67** [b] (39%)
	97%	 **63**	 **68** (47%) +  , 6-Ac **69**[c] (53%)
	98%	 **64**	 **70**[d] (24%)
	83%	 **65**	 **71** (60%)

R and R^1 as in **scheme 15**. a) Yield of cycloaddition step from scaffold **57**. b) Elimination of the tertiary alcohol occurred under acidic conditions used for acetonide deprotection originating two final products **66** and **67**. c) Since acetylation of the 6-position occured, two final products were obtained (**68** and **69)**. d) Decomposition lowered the yield.

Table 13: Methyl α-D-galactosides modified at the 2-position by click chemistry from the fully deprotected scaffold **58**.

Alkyne	Yield	**R**	Compound
	51%		**72**
	57%		**66**
	57%		**73**
	74%		**74**
	87%		**75**

R as in **scheme 16**.

The yields of the cycloaddition step using scaffold **57**, were good to excellent. However, the new entities had yet to be submitted to acidic conditions for the hydrolytic cleavage of the acetonide group, leading to the formation of side products. Elimination of the tertiary alcohol in **62**, acetylation of the 6-OH in **68** and decomposition of **70** were obtained.

The eleven methyl α-D-galactosides afforded either from scaffold **57** or **58**, were submitted to the competitive target-based assay for exploring the binding behavior towards H1-CRD.

1.20 Competitive target-based and Biacore assays (In collaboration with D. Stokmaier and Dr D. Ricklin from the Institute of Molecular Pharmacy, University of Basel).

Preliminary monitoring of the interactions between the methyl α-D-galactosides (**66** to **75**) and H1-CRD was performed by surface plasmon resonance (section **3.6**). The compounds were ranked according to their binding behavior and compared with the positive controls Gal and Gal*N*Ac and the negative control Glc (K_D > 20 mM). The ranking experiments were performed in parallel with the members of the hexitol-derivatives library. Further information to the data set normalization is reported in section **5.4.2**, SPR fits and details about the employed methodologies in **Annex 3** (provided by Dr D. Ricklin).

IC_{50} values and equilibrium dissociation constants (K_D) for reference compounds can be found in section **5.4.1** in *table 9*. Experiments were run under the same general conditions as for the hexitol-derivatives library.

IC_{50} values were determined with the competitive target-based assay (section **3.5**). All curves for IC_{50} determination of references and tested samples can be found in **Annex 2** (provided by D. Stokmaier).

Biacore preliminary ranking.

A preliminary ranking of the binding affinities of the methyl α-D-galactosides modified at the 2-position (**66** to **75**) towards H1-CRD was obtained by SPR. A single injection at a constant concentration (1 mM) was performed for each analyte giving preliminary binding information. Due to the negative SPR responses explained in section **5.4.2**, the binding affinities were normalized by the reciprocal of the MW (1/MW) (*figure 17*).

Six out of ten compounds analyzed bind either with lower or slightly better affinities than Gal. The exception is compound **69**. Some compounds (**67**, **72, 73**), need to be further analyzed as they generated positive signals in contrast to all other members

and references where negative responses were observed. In comparison with the hexitol-derivatives library (*figure 18*), all methyl α-D-galactosides studied, except **69**, were ranked worse than them. The exception to the rule, compound **69**, is the only member that is also substituted at the 6-position and therefore has a different general substitution pattern than all other compounds ranked.

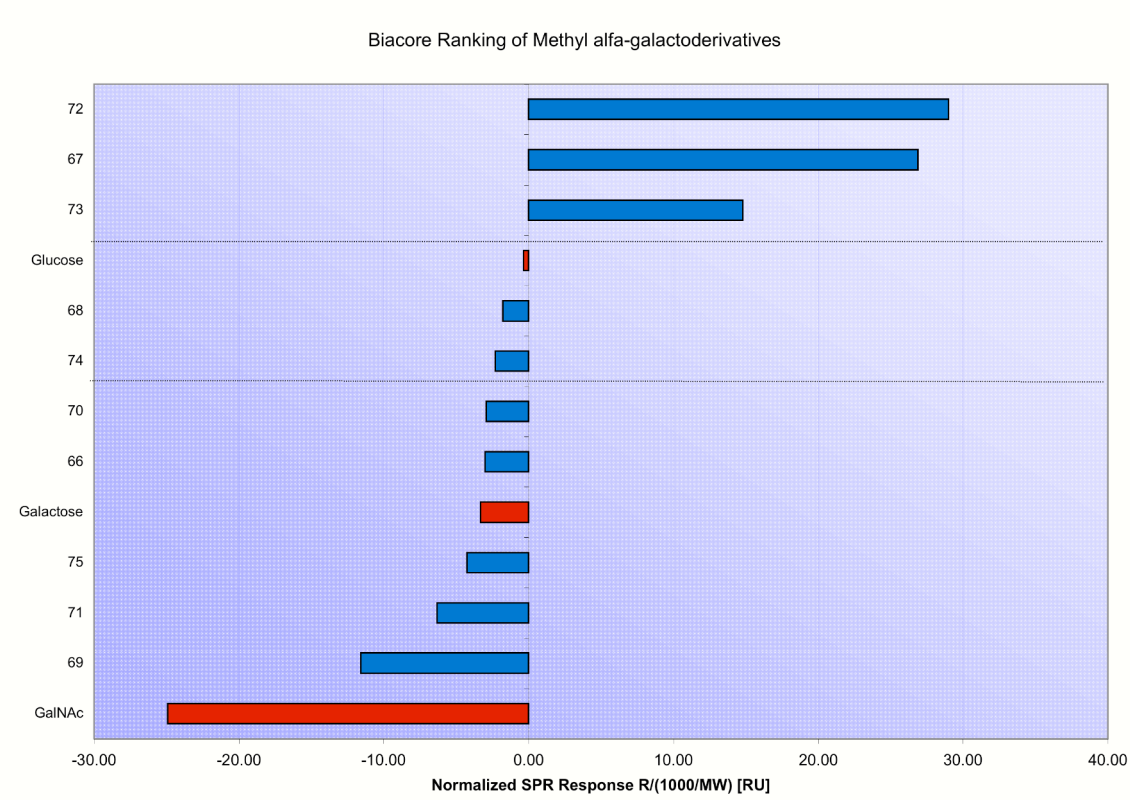


Figure 17: Biacore ranking of the binding affinities towards H1-CRD of the methyl α-D-galacto-derivatives modified at the 2-position. Comparison with reference compounds Gal, Gal*N*Ac and Glc was performed. The data was obtained for immobilized H1-CRD (aprox 8000 RU density equivalent to aprox 8 ng/flow cell) by performing a single injection at a constant concentration (1mM) per sample and was then normalized with the inverse of the corresponding analytes MW.

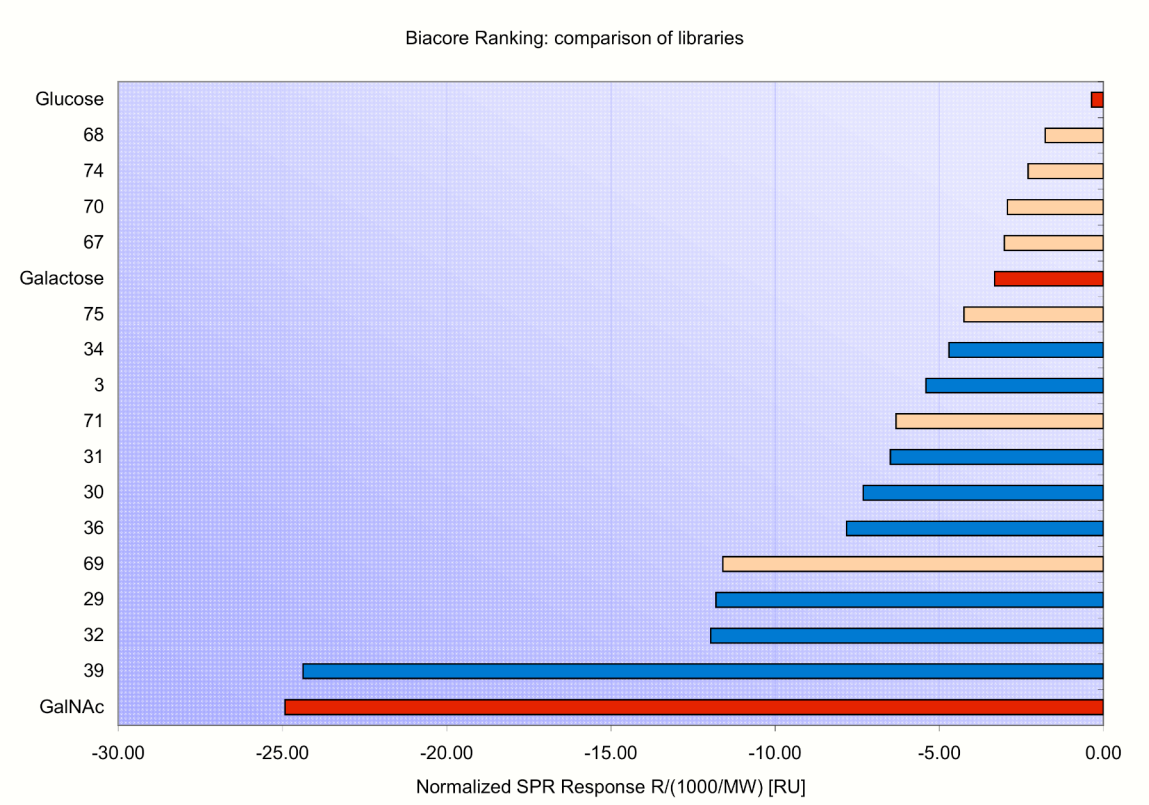


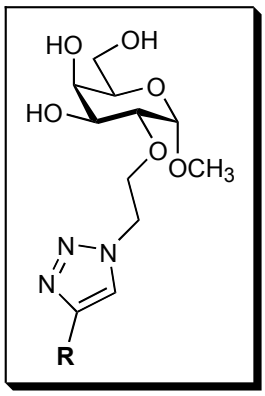
Figure 18: Biacore ranking of the binding affinities towards H1-CRD of methyl α-D-galacto-derivatives (yellow) and *lyxo*-hexitol (blue) libraries and reference compounds (red). The data was obtained for immobilized H1-CRD (aprox 8000 RU density equivalent to aprox 8 ng/flow cell) by performing a single injection at a constant concentration (1 mM) per sample and was then normalized with the reciprocal of the corresponding analytes MW. Positive signals were excluded from the compared rankings as they need to be further analyzed.

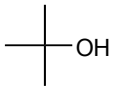
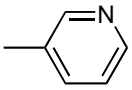
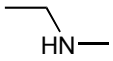
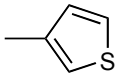
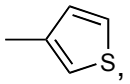
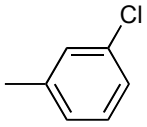
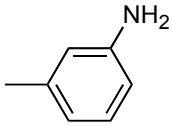
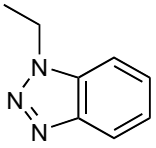
Competitive target-based assay.

IC_{50} values for the methyl α-D-galactosides were determined (*table 14*) by a competitive target-based assay. Due to the low affinities observed, no replicates were done unless unexpected values were obtained. As a consequence, the reported IC_{50} values should only be considered as preliminary.

Most compounds tested showed preliminary IC_{50} values that were of the same order of magnitude as methyl α-D-galactopyranoside. One possible explanation for the lack of improved affinity could be the steric factor leading to a clash with the receptor.

Table 14: IC_{50} (μM) values determined by competition target-based assay for methyl α-D-galactoside-derivatives modified at the 2-position.



Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
66		914	**71**		805
67		581	**72** [a)]		110 ± 13
68		952	**73**		399
69 [b)]	 6-Ac	592	**74**		1209
70		561	**75**		971

a) Value is the media ± standard deviation of two independent measurements b) same modification in position 2 as for compound **68** but acetylated in the 6-OH.

Biacore ranking *vs*. IC_{50} determination and further investigations.

Results from the ranking by Biacore and from the competitive target-based assay, for the methyl α-D-galactoside-derivatives **68** to **77**, were compared.

In general, the preliminary Biacore ranking of the binding affinities of the methyl α-D-galactoside-derivatives tested, predicted a lack of general improvement in the binding affinity towards H1-CRD relative to galactose. Such behavior was further sustained by preliminary IC_{50} values determined for the same derivatives. The chemical modifications performed did not translate into an improvement of the affinity for H1-CRD. However, they also do not interfere with the binding. An exception to this rule was compound **72** with a binding affinity of the same order of magnitude as Gal*N*Ac.

Of interest is the difference found between **68** and **69** in both assays. Both derivatives have the same substituent in position 2 but **69** has its 6-OH acetylated. The introduction of the acetyl group enhanced the affinity of **69**. A similar effect to this preliminary result was reported in the literature[35] for allyl α-D-Gal*N*Ac and its 6-*O*-methyl derivative. The introduction of this methyl group reduced the IC_{50} value approximately to its half as in the present case.

Compounds showing positive signals and **72** are further discussed.

Compound **72** was further analyzed by Biacore. Randomized triplicate injections of 8 concentrations between 6.0 mM and 2.7 μM were performed. A positive response with slow binding kinetics was obtained. However, the binding mode could not be fitted to a 1:1 model. The binding was nevertheless Ca^{2+} dependent, when Ca^{2+} was removed with EDTA no binding could be detected. Even if the binding is apparently specific, this molecule seems to bind in a completely different manner to H1-CRD. If the molecule binds near the binding site of the receptor, it might also avoid in the competition assay the binding of the competitor polymer, resulting in a low IC_{50} value.

Compound **67** was as well further analyzed by Biacore. Unfortunately, no good SPR fits were achieved possibly due to the presence of impurities. This compound was eliminated from the data set.

1.21 Compounds modified at the 2-position on solid phase.

Encouraged by the privileged situation of the substituents at the 2-position of Gal derivatives upon contact with H1-CRD, a new strategy for modification of the monosaccharides moieties was planned. Furthermore, to determine if a combined effect of the substituents does have an added impact on the overall affinity, a method which later on could also be used on solid phase to introduce diversity at the 2- and 6-positions of Gal derivatives in a combined way was developed. In addition, the robustness of the linker under the required reaction conditions was assessed.

The galacto-scaffold for solid phase was chosen as for allowing amino acid couplings at its 2-position. The scaffold was in addition developed as a 6-azidosugar for introducing later the 2,6-disubstituted combined strategy.
On one hand, these are high yielding reactions very much employed on solid phase. On the other hand, a 2-*N*-acyl *galacto* moiety is expected to maintain some of the characteristics that Gal*N*Ac has for an increased selectivity to our protein[32,34] and to interact with His 256 which is responsible for the preferential binding of GalNAc relative to Gal,[32,33]. Moreover, by varying the length of the chain coupled to the 2-amino group, it is possible to study the optimal situation for the interaction with the receptor.

Synthesis of the scaffold for solid phase strategy.

The scaffold of choice had to fulfill a number of requirements for achieving the objectives of this strategy. First, a 2-deoxy-2-amino-galactosamine derivative was needed and therefore a protection for the nucleophilic amino group was developed. Second, the new *galacto* moiety was planned as a 6-azidosugar for modifications that were later introduced. Third, a selective synthesis for obtaining only the β derivative (in general, enhanced affinity towards H1-CRD than its α counterpart) was designed.

The synthesis was started from glucosamine hydrochloride available in large amounts at our facilities. It offered an elegant approach for the synthesis of the galactosamine derivative for which an epimerization was required to obtain the axial orientation of the 4-OH.

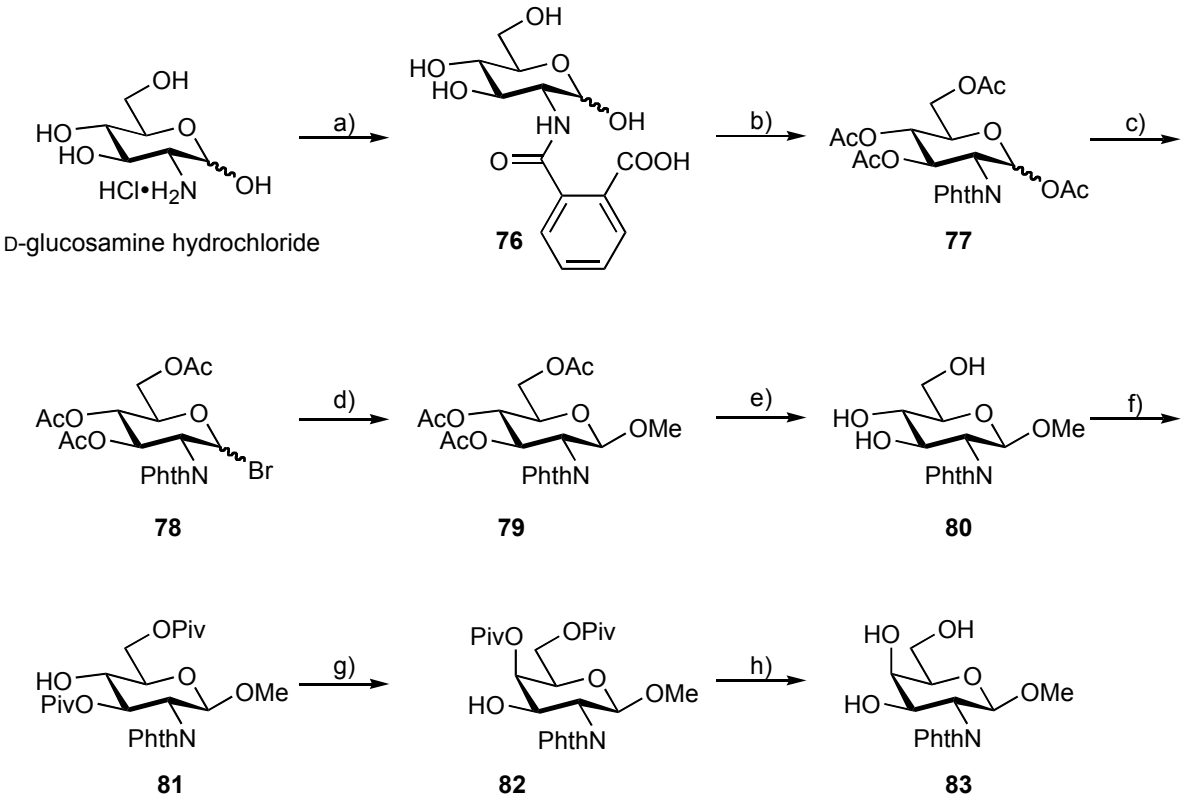
1.21.1.1 Synthesis of the galactose epimer

The β selective oriented synthesis and the epimerization process can be seen in *scheme 17*. The 2-amino group of glucosamine was *N*-blocked with the basic labile phthaloyl group. For this purpose glucosamine hydrochloride was smoothly converted by phthalic anhydride in aqueous dioxane into its *O*-carboxybenzoyl derivative (**76**) and hence by acetic anhydride with sodium acetate as basic catalyst in 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phtalimido-D-glucose (**77**).[45,83] Having the α face of the sugar partially blocked, the formation of the anomeric bromide by use of 33% HBr in AcOH, gave mainly the β-anomer (α:β = 1:4) as indicated by ^{1}H NMR. Clearly the two anomeric centers with the characteristics coupling constants, $J_{1,2}$ = 3.7 Hz for the α-anomer and $J_{1,2}$ = 9.6 Hz for the β-anomer, could be assigned. Due to the instability of brominated compounds, the product, quantitatively afforded, was submitted immediately to the next step without further purification. Methyl β-D-glucopyranoside was obtained by the Koenigs-Knorr reaction with employment of silver carbonate on the poly-*O*-acetylglucosyl bromide.[84] In this opportunity β selectivity was achieved in very good yield (87%). ^{1}H NMR showed a single anomeric center with coupling constant $J_{1,2}$ = 8.4 Hz characteristic for the β isomer.

In order to epimerize C-4 the method of Cipolla *et al.* was employed.[85] The epimerization was achieved taking advantage of acyl migration in basic media preferring an axial configuration to an equatorial one. After deacetylation under Zemplén conditions, the pivaloy ester was introduced at positions 3 and 6 selectively. The selective character of the pivaloylation can clearly be deduced from the analytical data. Protons H-3 and H-6 suffered a dramatic shift to lower fields in the ^{1}H NMR of **80** compared to **81** (**80** → **81**: H-3 δ 4.27 ppm → 5.59 ppm, H-6 δ 3.88 ppm → 4.45 ppm). The resulting compound **81** was converted into the corresponding triflate derivative by triflic anhydride.[86] Immediate displacement with water gave the

expected epimer **82** in 59% yield. Comparison of the H-4 signals in the ^{1}H NMR spectrum of the initial *gluco* form and the epimer **82** shows a clear shift to lower fields of the mentioned protons (H-4: **81** → **82**: δ 3.59 ppm → 5.34 ppm) indicating the presence of the pivaloyl protecting group at the 4-position consequence of its migration.[87] However, due to lack of good resolution in the ^{1}H NMR spectrum recorded for compound **82** the characteristic doublet of the *galacto* H-4 could not be well observed. The signal appeared instead as a broader peak assigned as a multiplet. Nevertheless, the difference with the *gluco* moiety was clear. The H-4 of the *gluco* form **81** showed in its ^{1}H NMR spectrum a triplet with a big coupling constant $J_{3,4}$ = 8.9 Hz. In contrast, derivatives obtained from **82** had small coupling constants between protons H-3 and H-4 (2.8 Hz - 3.2 Hz) characteristic for the *galacto* epimer. For derivatives where the doublet could not be seen, the coupling constant was obtained from the H-3 signal.

The newly obtained galactose derivative **82** was then deprotected to obtain methyl 2-deoxy-2-phtalimido-β-D-galactopyranoside (**83**). The basic conditions required for the removal of the pivaloyl groups produced the opening of the phthalimido ring that had to be closed once again as in step b) (*scheme 17*). This procedure caused the acetylation of the hydroxyl groups that could be deprotected under only slightly basic conditions, resisted by the phthalimido protecting group.



Scheme 17: a) Phthalic anhydride, $NaHCO_3$, H_2O/dioxane (4:3), r.t., 4 days;[83] b) NaOAc, Ac_2O, 140°C, 20 min, (57% a) and b));[83] c) HBr/AcOH, DCM, r.t., 5h (quant.);[83] d) Ag_2CO_3, $CaSO_4$, MeOH, r.t., 23 h (87%); e) NaOMe, MeOH, r.t., 12 h (quant.); f) PivCl, DMAP, Py, -38°C, 19 h (67%); g) i) Tf_2O, DMAP, DCE/Py (2:1), 0°C, 17 h, ii) H_2O, 80°C, 5 h (59%);[85] h) i) NaOMe (0.5 M), MeOH, r.t., 12 h, ii) NaOAc, Ac_2O, 140°C, 15 min, iii) NaOMe, MeOH, r.t., 12 h (quant.).

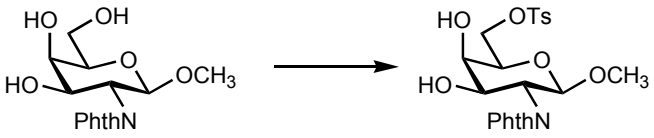
1.21.1.2 Functionalization of the scaffold.

Methyl 2-deoxy-2-phtalimido-β-D-galactopyranoside (**83**) had to be properly functionalized to obtain a scaffold suitable for the modifications planned on solid phase. In addition, the amount of scaffold obtained in this strategy is not trivial. For quantitative loading of each lantern a large excess of material is required (3.3 eq.). The modular nature of the lanterns employed as polymer support, suppose a disadvantage for the minimal amount of material required for loading one unit. As a consequence, it was developed a synthetic pathway with preferred overall higher yield in spite of the number of steps required for it.

To transform **83** into a 6-azidosugar, a very good leaving group such as tosyl could be ideally selectively introduced, to then be displaced by the azido function. Two

different methods employing pyridine or dibutyltin oxide catalysis[88] were tested for this purpose (*table 15*). Either due to lack of selectivity or selective but insufficient yield of the 6-tosylated product, the overall yield obtained was still not satisfactory for the purpose of this work. Other alternatives were studied to improve the overall yield in spite of the additional steps required.

Table 15: Results of tosylation procedures on compound **83**.

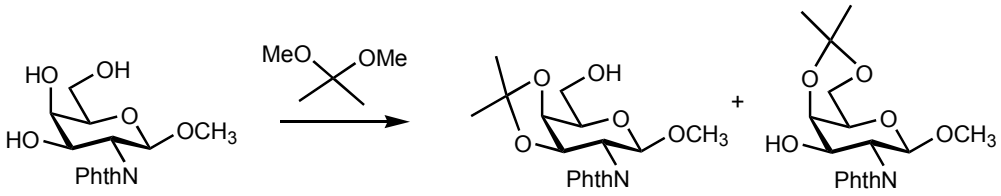


83 **84**

TsCl	Solvent	Other cond.	Temperature	Time	Yield
1.5 eq	Py	_	r.t.	20 h	16%[a)]
1.0 eq	DCM	Bu_2SnO, Et_3N[88]	r.t.	4 h	60%[b)]

a) Many polytosylated products were obtained. b) 60% of 6-monotosylated product **84** obtained considering the starting material that was recovered. Other bi-tosylated products obtained as well.

A second alternative, including tosylation but on a protected derivative was considered. The aim was to simultaneously protect the 3,4-OHs with the acetonide group followed by tosylation at the 6-position with subsequent cleavage of the acetonide group. The selective protection of the 3- and 4-positions of the fully deprotected methyl β-D-galactopyranoside with the acetonide group is well known[72] and was also corroborated by myself (compound **12**). However, a mixture of the 3,4- and 4,6-protected derivatives (*scheme 18*) was in every case obtained (*table 16*) with predominance of the non-desired later product. The bulky phthalimido group might be the cause resulting in steric hindrance around the 3-OH. This second alternative could therefore not be employed.



83 **85** **86**

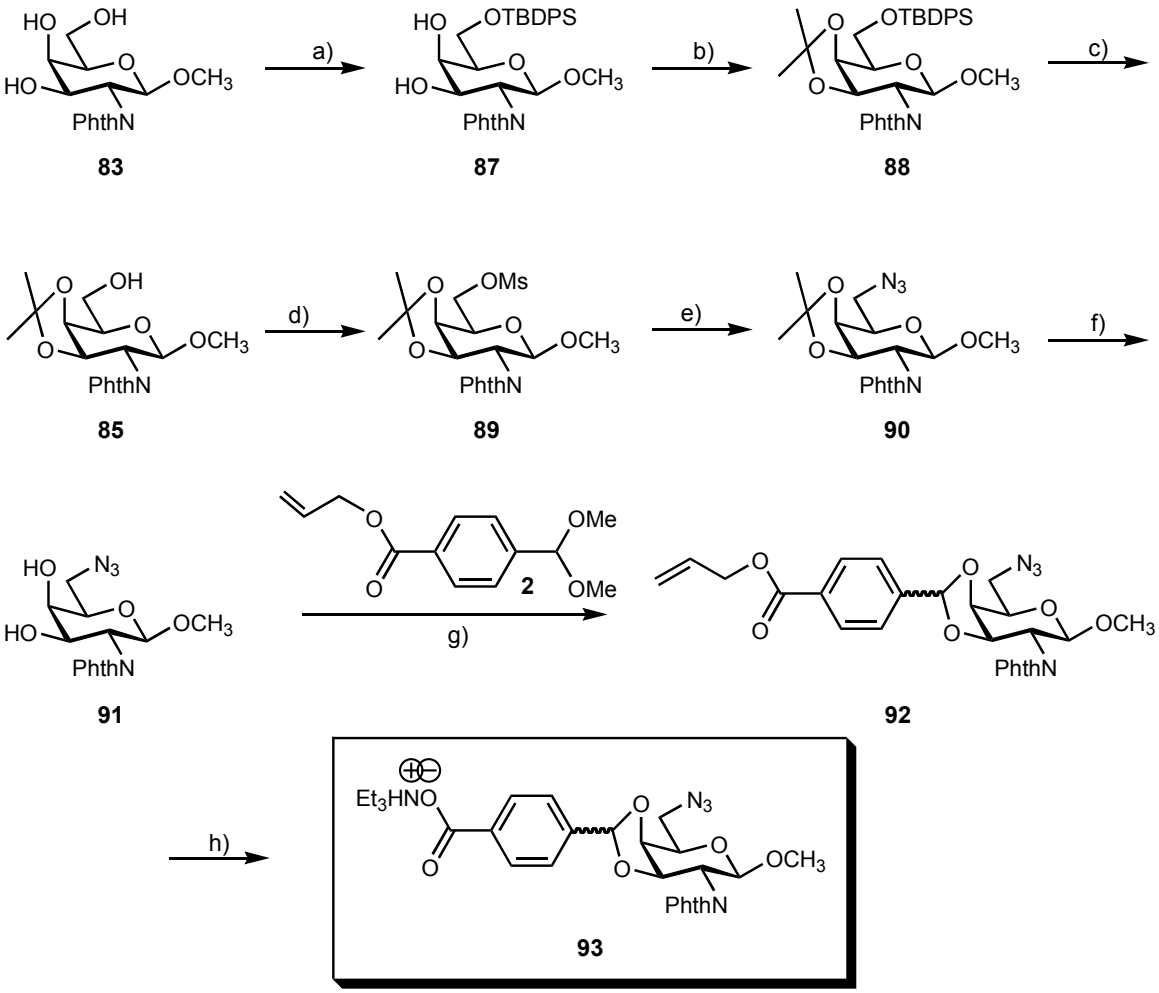
Scheme 18: Lost of selectivity for the acetonide introduction at the 3,4-OHs on the 2-deoxy-2-phtalimido derivative **83**.

Table 16: Lost of selectivity for the acetonide introduction at the 3,4-OHs on the 2-deoxy-2-phtalimido derivative **83**.

2,2-dimethoxypropane	Solvent	catalyst	Temp.	Time	**85**:**86**
1.5	DMF	*p*-TsOH·H_2O	80°C	7 days	1:6
1.5	DMF	*p*-TsOH·H_2O	80°C	2.5 h	1:3
1.5	CH_3CN	CSA	r.t.	55 h	100% **86**

The third attempt to achieve a better overall yield of the entire synthesis was the one finally applied (*scheme 19*). Three subsequent protecting and deprotecting steps resulting in 78% of overall yield were applied to obtain the mesylated derivative **89** (95%), transformed into the desired azido compound **90** with 96% yield. After final acetonide deprotection (quantitative), **91** could be coupled with the allyl-protected linker **2**. Allyl removal exposed the free carboxylic acid required for coupling with the aminomethylated lanterns.

The overall yield could be improved by 14% and short reaction times and easy purifications were possible.



Scheme 19: a) TBDPSiCl, Et_3N, DMAP, DCM, r.t., 12 h (96%); b) 2,2-dimethoxypropane, *p*-TsOH·H_2O, CH_3CN, r.t., 16 h (96%); c) Bu_4NF, THF, r.t., 3 h (85%); d) MsCl, Py, 0°C to r.t., 2.5 h (95%); e) NaN_3, 15-C-5, DMF, 100°C, 5 days (96%); f) 80% aqueous AcOH, 80°C, 2 h, (quant); g) **2**, *p*-TsOH·H_2O, CH_3CN, 105°C, 66 h, (64%); h) Pd(0)$(PPh_3)_4$, pyrrolidine, *N*-methyl morpholine, THF, 0°C, 30 min[89], column with Et_3N, (86%).

Monosaccharide mimic **93** adequately functionalized for performing amino acid couplings at the 2-position and 1,3-dipolar cycloadditions at the 6-position on solid phase, was obtained from glucosamine hydrochloride in 18 synthetic steps with an overall yield of 8%. A more elegant alternative synthetic pathway via the 6-monotosylated compound **84** was also developed and represents an alternative method for the production of the final compound with a relatively lower yield.

1.21.1.3 Cleavage of the allyl group.

The various functionalities present on the carbohydrate mimic **92** made it difficult to find a selective method for the removal of the allyl-protecting group. Several methods were tried for its cleavage (*table 17*). Experiments were performed using different starting materials or a combination of them as to simulate the situation on compound **92**.

First, basic conditions employing LiOH were examined. Allyl removal was previously performed under these conditions and can be found in *scheme 12*, section **5.3.2**. Compound **88** was submitted to the mentioned cleaving conditions, but the phthalimido group was as a result cleaved. Moreover, if less equivalents of LiOH were employed as to avoid the loss of the phthalimido group, no cleavage of the ally ester from compound **2** could be afforded.
Second, potassium *t*-butoxide in dimethyl sulphoxide (DMSO)[59,90-92] and THF was employed for isomerization of the ally to its labile 1-propenyl form. No cleavage of the allyl group was achieved.
Third, the Wilkinson catalyst was used for the isomerization of the allyl ester. A combination of an allyl ester containing moiety (**6**) and an azido sugar (**58**) were employed for reproducing the situation of compound **92**. Tris(triphenylphosphine)rhodium(I)-chloride with temperature and at room temperature but in presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) were tested.[58,63,93] The removal of the protecting group was still not satisfactory in the best of the cases. Nevertheless, this experiment showed that the phosphine, probably because it was coordinated to the transition metal, did not affect the azido functionality of **58**.
Finally, the cleaving method of choice involved the use of tetrakis(triphenylphosphine)palladium(0) ($(PPh_3)_4Pd(0)$) with participation of a nucleophilic base as scavenger. The selected method, shows in entries 7 and 8 of *table 17* to cleave the allyl ester and allowed the quantitative recovery of the azido-containing compound **50** used for the test.[63]

Table 17: Conditions tested for the removal of the allylic ester of compound **92**. Experiments were performed using different starting materials or a combination of them as to simulate the situation on compound **92** with respect to other functionalities. The precious material 94 was not consumed.

Entry	Starting material	General conditions	Temp	time	Result
1	**88**	LiOH 10 eq Dioxane/H_2O (1:1)	r.t.	22 h	Phth opening
2	**2**	LiOH 1.5 eq Dioxane/H_2O (1:1)	r.t.	24 h	n.c.
3	**6**	i) KtBuOH, DMSO ii) HgO/HgOAc, Acetone: H_2O (10:1)	i)100°C ii) r.t.	i) 15 min ii) 2 h	n.c.
4	**6**	i) KtBuOH, THF ii) HgO/HgOAc, Acetone: H_2O (10:1)	i) 60°C ii) r.t.	i) 24 h ii) 5 days	n.c.
5	**6 + 58**	$(PPh_3)_3RhCl$ (0.1 eq) EtOH:H_2O (9:1)	i) 70 °C ii) r.t.	40 h	35%
6	**6 + 58**	$(PPh_3)_3RhCl$, DABCO EtOH:H_2O (9:1)	r.t.	14 h	n.c.
7	**6**	$(PPh_3)_4Pd(0)$, NMM, pyrrolidine,THF	0°C	30 min	quant.
8	**50**	$(PPh_3)_4Pd(0)$, NMM, pyrrolidine, THF	0°C	30 min	quant. recover

n.c. means “no cleavage”

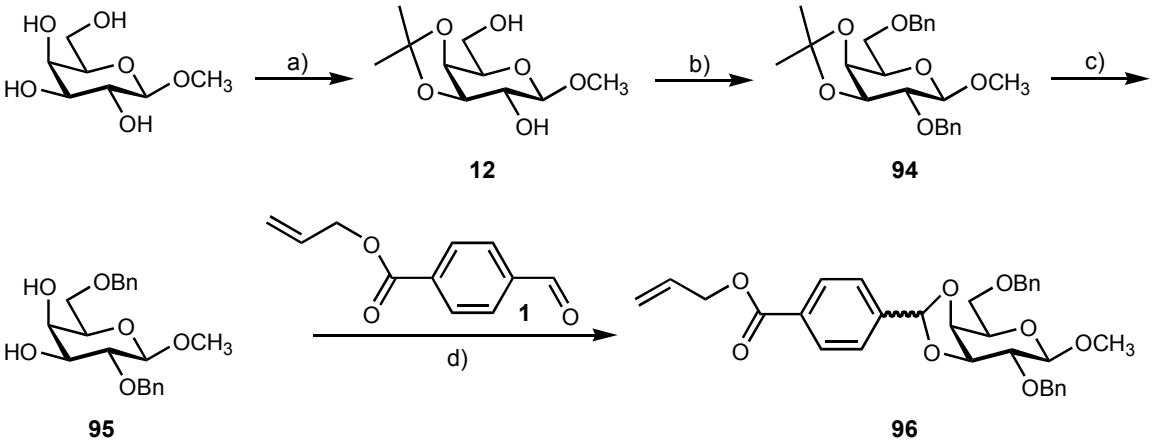
Solid phase tests required for modifications at the 2-position.

To assure the lasting life of scaffold **93** on the solid support and the correct evolution of the experiments, few analyses were carried out in solution.

a) Stability experiments

Two stability tests under different handling conditions of intermediate steps were performed to study the stability of the connection between linker and scaffold. The main interest was to study the stability towards amino acid coupling conditions as well as Fmoc-deprotection conditions. To avoid consuming **93**, which is a very expensive material in terms of effort and time, a new intermediate was synthesized in only 4 steps from methyl β-D-galactopyranoside (*scheme 20*).

Derivative **96** was easily synthesized and the two isomers could be separated and tested independently. After selective 3,4-*O*-isopropylidenation (quantitative), the 2- and 6-hydroxyls were benzylated with NaH and benzylbromide (63%). Subsequent removal of the acetonide provided the 3,4-OHs free to be coupled with the linker **1** to form the benzylidene acetal linkage for stability studies.



Scheme 20: a) 2,2-dimethoxypropane, *p*-TsOH·H_2O, DMF, 80°C, 4 h (quant.);[72] b) NaH, BnBr, DMF, 0°C-r.t., 4 h, (63%); c) 80% aqueous AcOH, 80°C, 3 h, (94%); d) **1**, *p*-TsOH·H_2O, r.t., 24 h, (63%).

First, stability studies for the benzylidene acetal linkage under the Fmoc-deprotection conditions were carried out. The amount of material that would be possible to load on

one lantern for each isomer was separately dissolved in 20% piperidine solution in DMF. The solutions were followed at different intervals of time by HPLC. At intervals of 10 min during the first 30 min, and then 2 h after the beginning of the test, aliquots of the solutions were taken and diluted with DMF. 10 μL from the resulting 1-2 mg/mL solutions were injected in the HPLC. No changes were detected in the chromatograms or in the UV spectrums of the peak detected. The benzylidene acetal linkage resisted at all tested times the conditions required for Fmoc-deprotection.
Second, the stability of the benzylidene acetal linkage was investigated in a similar way for standard amide bond formation. Both isomers of **96** were separately dissolved in DMF and diisopropylcarbodiimide (DIC) and hydrate-1-hydroxybenzotriazole (HOBt) were added as coupling reagents. The test was executed in solution with the amounts required as for one lantern. In parallel, a solution containing only DIC and HOBt in DMF was also closely followed. As references the corresponding isomers of **96** were used as well as HOBt and DIC. Aliquots of the test solutions were taken at different time intervals, diluted with DMF, analyzed by HPLC and compared with the corresponding references. **96** showed to be stable during the testing period (72 h) for the condition tested.

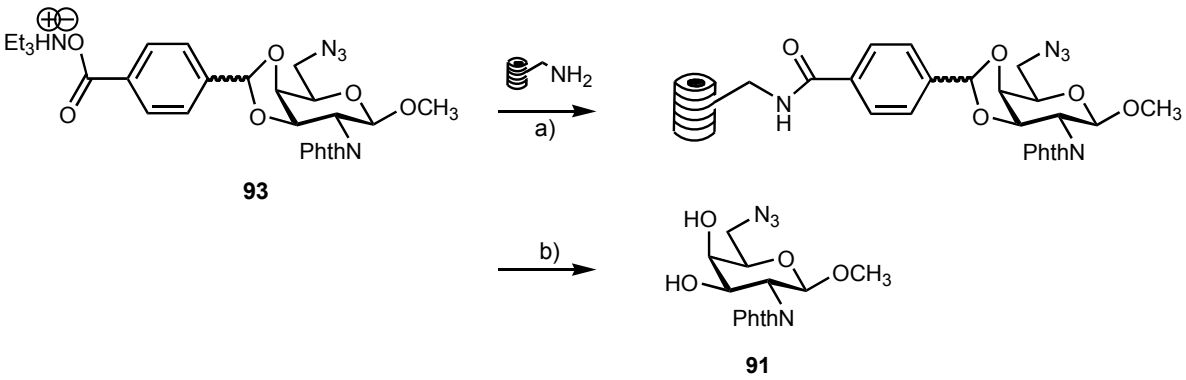
In addition, similar developed experiments to show the versatility of the selected linker were performed. The linkage under study proved to be stable under basic conditions like large excess of diethylamine, or tetrabutylammonium fluoride. This fact can provide flexibility in modifications to be planned for solid phase with this linker. It would be possible to work with acid labile protecting groups in the presence of the similarly acid labile benzylidene acetal.

b) Loading experiment.

This specific experiment had to be carried out with **93**, the scaffold to be modified on solid phase. The aim was to test the quantitative character of the loading and cleavage procedures, a prerequisite for working with the solid support.

Due to the aminomethylated nature of the lanterns, a standard coupling procedure using DIC as activating agent in the presence of HOBt for OBt-ester formation, resulted in a satisfactory loading protocol.[75] More than 92% of the material loaded in

the lantern could be recovered after hydrolytic acid cleavage of the acetal linkage, and the excess of **93** used could also be successfully retrieved. (*scheme 21*)



Scheme 21: a) i) DMF, r.t., 30 min; ii) 20% Et_3N, DMF, r.t. 3 x 10 min, iii) HOBt, DIC, DMF, r.t., 15 h;[75] b) 80% aqueous AcOH, 2% TFA, r.t., 2 x 15-20 h.[55]

Amino acid coupling on solid phase.

Since the pioneering work of the Nobel laureate Merrifield in 1963, every step in the peptide synthesis process has been scrutinized and optimized to produce high yielding reactions. Many improvements in conditions and reagents transformed the peptide synthesis into a reliable procedure.

The conditions used for coupling amino acids to the immobilized 2-deoxy-2-amino sugar mimic are based in the same chemical principle as the solid phase peptide synthesis. After phthalimido deprotection of the amino group on the immobilized **93**, an amide bond is formed by condensation of the afforded free amine with the carboxylic acid of an Fmoc-protected amino acid. Due to the low reactivity of the reaction partners and the establishment of a equilibrium, the carboxylic acid needs to be activated for achieving satisfactory yields. Many different activating reagents are available and for each case the best options should be found as to obtain the best outcome.

Three different coupling procedures were tested in parallel on solid phase trials as to select the best option for the present case.

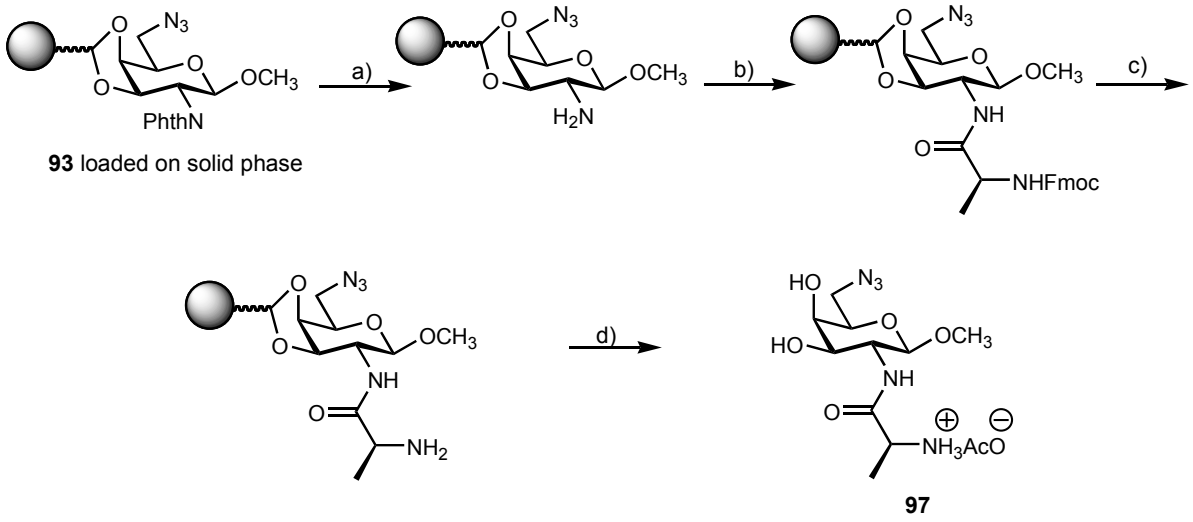
First, one of the most popular *in situ* activating reagents DIC was used in combination with HOBt.[94]
Second, HBTU (2-(1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) assisted coupling in the presence of HOBt, for the generation of the OBt ester was performed.[95]
The third method employed was a PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium-hexafluorophosphate) mediated condensation.[96] PyBOP is a standard *in situ* coupling reagent employed also in combination with HOBt.
The last two methods also require the use of a tertiary base as for example diisopropylethylamine (DIPEA)[97]. DMF was in every case the solvent of choice.

In a first attempt, after deprotecting the phthalimido group with hydrazine in MeOH, the lantern was left to react overnight with alanine. Analysis of the cleaved material by MS and ^{1}H NMR showed a mixture of two products (data not given). To elucidate the composition of the mixture, the crude material was acetylated and only then the products obtained separated by silica gel chromatography. It was then clear that the amino acid-coupled and non-coupled materials were present in the original mixture in a ratio 0.6:1. Even though the phthalimido deprotection was complete, one cylce of amino acid coupling gave only 38% of the expected product. Several cycles of coupling procedures had to be employed to achieve completion, a standard procedure in solid phase synthesis. After different attempts it was found that five coupling cycles were necessary to drive reactions to completion. Alanine was used as Fmoc-protected amino acid for all the trials.

After five coupling cycles, all three methods provided only the expected product. Recovery of the loaded material was satisfactory in every case (81% to 95%). In the presence of HBTU, fewer impurities were detected by ^{1}H NMR spectroscopy and was the reason for selecting it as the method of choice (*scheme 22*).

After activation of the solid phase (provided as trifluoroacetate salt) with triethylamine, **93** was loaded using a 3-fold excess of it (*scheme 21*) and standard coupling conditions mediated by DIC/HOBt . First, the phthalimido group was cleaved with hydrazine monohydrate in methanol using repeated cycles. Second, an Fmoc-protected amino acid was coupled to the 2-amino sugar by HBTU assisted process.

In every step, excess of reagents were employed for driving the reactions to completion. Third, the Fmoc group was easily cleaved under mild conditions with an amine base. Finally, the target product was cleaved from the solid phase by acid hydrolysis of the benzylidene acetal linkage (*scheme 22*). The acetate form of the product was obtained. Reversed phase purification with a gradient of 5% MeOH in H_2O gave the expected final products.



93 loaded on solid phase

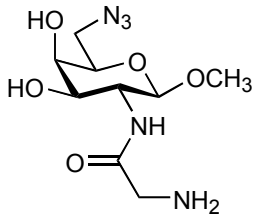
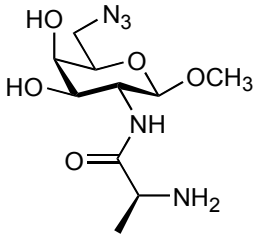
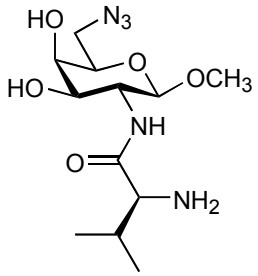
97

Scheme 22: a) $H_2NNH_2 \cdot H_2O$, MeOH, 80°C, 4 x 3 h, 1 x 12 h[98]; b) Ala-Fmoc, HBTU, HOBt, DIPEA, DMF, r.t., 3 x 4 h, 2 x 12 h; c) 20% Piperidine, DMF, r.t., 3 x 10 min; d) 80% aqueous AcOH, 2% TFA, r.t., 2 x 15-20 h.

Compounds modified at the 2-position on solid phase.

Few compounds were synthesized following the procedure of *scheme 22*, to determine the impact of the modifications of choice on the binding affinity for H1-CRD (*table 18*). Glycine, Alanine and Valine were the amino acids selected.

Table 18: Compounds synthesized on solid phase modified by amino acid coupling at the 2-position of a methyl β-D-galactosamine mimic.

Amino acid	Compound	Structure	Recovery
Gly	**98**		a)
Ala	**99**		81% b)
Val	**100**		100% b)

a) Decomposition occurred when concentrating at 40°C under reduced pressure. Colorless to yellowish color change was observed. The residue after evaporation represented 100% of the material loaded, however, after purification only 20% of the material could be obtained. b) Purity equal or higher to 98% according to ^{1}H NMR.

1.22 Competitive target-based and Biacore assays (In collaboration with D. Stokmaier and Dr. D. Ricklin from the Institute of Molecular Pharmacy, University of Basel).

Competitive target-based assays (section **3.5**) as well as SPR experiments (section **3.6**) were carried out with the compounds modified by amino acid coupling (**98** to **100**). These experiments allowed evaluating the impact of these specific

modifications on the affinity for H1-CRD. The use of the two complementary analyses offered highly reliable data.

Competitive target-based assay.

IC_{50} values for compounds **98** to **100** (*table 18*) were determined with the competitive target-based assay (*table 19*). Unless specified, duplicates were performed with good reproducibility.

All three compounds modified at the 2-position by amino acid coupling showed increased affinities towards H1-CRD compared to methyl β-D-galactopyranoside. The best inhibitor found was the alanine derivative **99**, which competed approximately 20-fold more effectively than Gal, and 12-fold with respect to methyl β-D-galactopyranoside for binding to the H1-CRD. The IC_{50} value of **99** was nearly half (1.7 times better) of that of Gal*N*Ac.

Table 19: IC_{50} (μM) of methyl 6-azido-β-D-galactosamine derivatives modified at their 2-position.

Compound	Amino acid	IC_{50} (μM)
98	Gly	401 [a]
99	Ala	46 ± 9
100	Val	385 ± 61
Gal	-	1754 ± 57
Me β-D-Gal	-	545 ± 39
Gal*N*Ac	-	78 ± 5
Me 6-azido-α-D-Gal	-	1200 [b]
46		248 ± 41

Values are the media ± standard deviation of two independent measurements. a) Only one determination performed. b) Data from Lee *et al.*,[38] IC_{50} value determined for isolated rabbit lectin for methyl 6-azido-α-D-galactopyranoside.

Lee *et al.*[38] determined in competitive assays IC_{50} values for the isolated rabbit lectin. It was showed in the present work, the close relationship between the IC_{50} values obtained for both receptors; H1-CRD and the rabbit lectin (*table 9*). Methyl 6-

azido-α-D-galactopyranoside showed in the experiments of Lee and co-workers[38] no improvement in the binding affinity as a consequence of the introduction of the azido group at the 6-position. In our collected data, the azido derivative **46** (1,5-anhydro-6-azido-2-deoxy-D-*lyxo*-hexitol) showed an important increase in the binding affinity compared to the IC_{50} value of 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol (**3**). Further studies to evaluate the real influence of the 6-azido group will still be performed.

Biacore assays.

Biacore determinations of equilibrium dissociation constants (K_D) of compounds **46** and **98** to **100** were carried out (*table 20*). Gal, methyl β-D-Gal and Gal*N*Ac were used as reference compounds.

Table 20: Equilibrium dissociation constants K_D (μM) of methyl 6-azido-β-D-galactosamine derivatives modified at their 2-position by amino acid coupling.

Compound	Amino acid	K_D (μM)
98	Gly	1120
99	Ala	134
100	Val	1268
Gal	-	2080
Me β-D-Gal	-	1780
Gal*N*Ac	-	112
46	-	855

Randomized triplicate injections of 8 concentrations (three fold serial dilutions) between 6.0 mM and 2.7 μM were performed.

All compounds tested in Biacore showed an increased affinity for the receptor with respect to Gal and methyl β-D-galactopyranoside. The binding affinity of compound **99** was of the same order of magnitude as Gal*N*Ac while for the other two *N*-acyl derivatives measured only a two-fold improvement in the binding affinity was detected relative to Gal. In the case of the *galacto*-mimic **46** (1,5-anhydro-6-azido-2-deoxy-D-*lyxo*-hexitol), the binding affinity for H1-CRD was two times better than for

methyl β-D-galactopyranoside. The differences recorded between compounds can be appreciated in *figure 19* where the response obtained for each analyte was plotted against the concentration. In the first set of graphs, the responses were normalized with the reciprocal of the MW (due to negative SPR responses) of the corresponding compounds (section **5.4.2**). In the second set of curves (*figure 19*) the normalization was done considering also the maximum response achieved. Only by doing so did it become clear for all the analyzed compounds that there is a parallel shift towards lower concentrations of the inflection point of the curves. The high similarity in binding behaviour between Gal*N*Ac and **99** can also be observed.

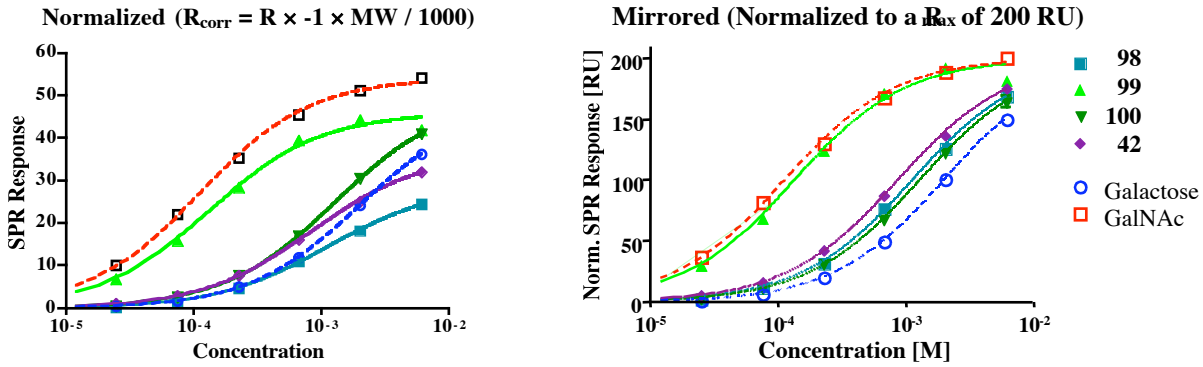


Figure 19: Curves of SPR response vs. concentration. Normalization by the reciprocal of the MW was done in the first set of curves and also to the maximal response, where the saturation is achieved, in the second set. Measurements were performed in 10 mM HEPES buffer with 50 mM $CaCl_2$, pH 7.4 with 3% DMSO. Randomized triplicate injections (three fold serial dilutions) between 6.0 mM and 2.7μM were done.

Biacore ranking *vs*. IC_{50} determination.

IC_{50} values determined using the competitive target-based assay were compared to the equilibrium dissociation constants (K_D) determined by Biacore experiments (*table 21*, *figure 20*).

Table 21: IC_{50} (μM) values determined by competitive target-based assay and equilibrium dissociation constants K_D (μM) determined by SPR for the methyl 6-azido-β-D-galactosamine derivatives modified at their 2-position by amino acid coupling.

Compound	Amino acid	IC_{50} (μM)	K_D (μM) [b]
98	Gly	401 [a]	1120
99	Ala	46 ± 9	134
100	Val	385 ± 61	1268
Gal	-	1754 ± 57	2080
Me β-D-Gal	-	545 ± 39	1780
Gal*N*Ac	-	78 ± 5	112
46	-	248 ± 41	855

IC_{50} values are the media ± standard deviation of two independent determinations except for a) in which only one measurement was performed. b) Obtained from randomized triplicate injections of 8 concentrations (three fold serial dilutions) between 6.0 mM and 2.7 μM.

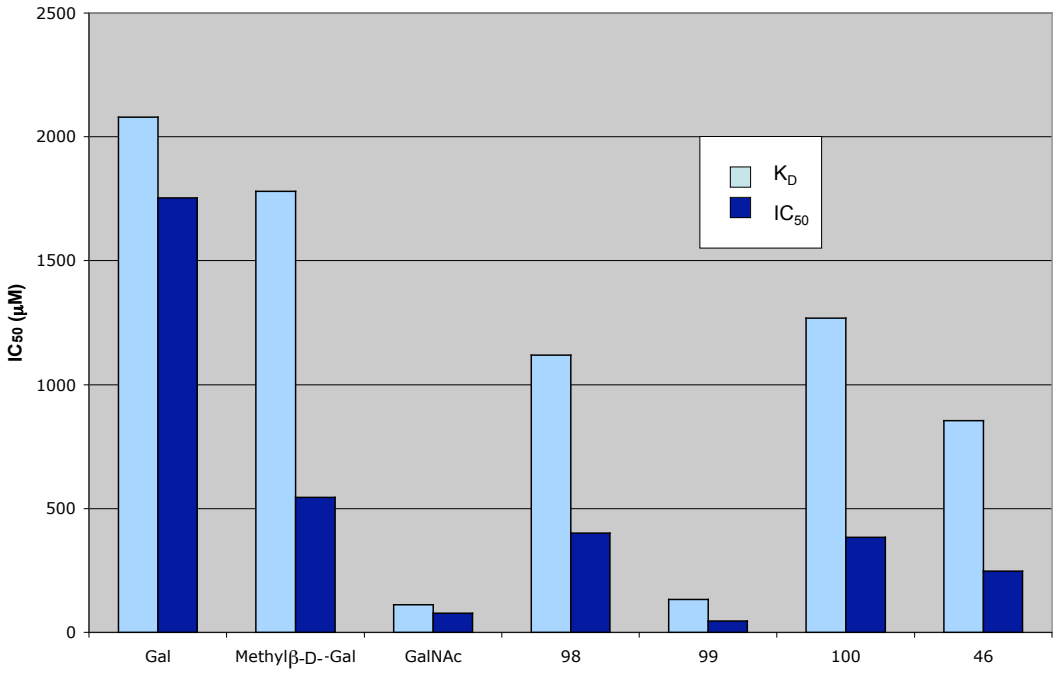


Figure 20: Graphic comparison of IC_{50} (μM) values and equilibrium dissociation constants K_D (μM) for methyl 6-azido-β-D-galactosamine derivatives modified at their 2-position with amino acids. Relevant reference compounds are included. Same batch of H1-CRD was employed in both experiments. Standard deviations are not graphically represented.

All K_D values obtained by SPR were higher than the corresponding IC_{50} values determined with the competitive target-based assay. Values generated by Biacore were always 2-3 times higher than by competitive assay. However, the general monosaccharide ranking, when the standard deviations were considered, was consistent in both experiments.

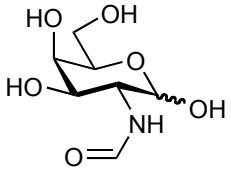
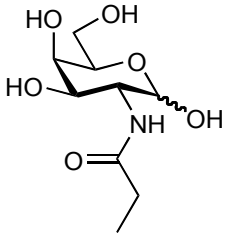
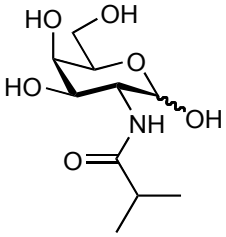
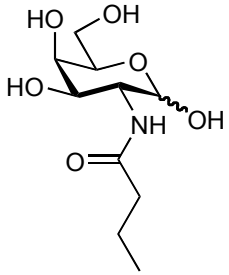
The competitive target-based assay proved to be more sensible to the changes than Biacore for molecules with improved affinities relative to Gal but still in the high or medium μM range (IC_{50} of 300-500 μM). However, when a real improvement in the binding affinity occurs, like in the case of **99**, both experiments measured it similarly. Compound **99** reached binding affinity values in the low μM range, very similar to Gal*N*Ac.

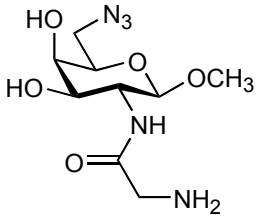
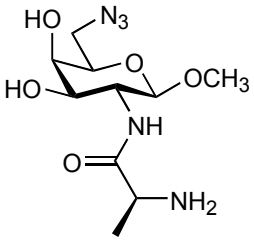
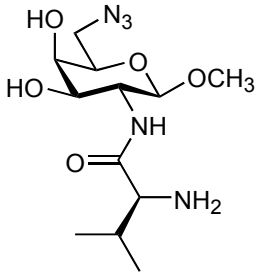
Using instead methyl β-D-galactopyranoside as reference compound, differences between the binding affinities determined for a given analyte in both experiments (Biacore and competitive-assay) were conserved. Methyl β-D-galactopyranoside is

still a weak antagonist (mM range) but 2-3 times better than Gal allowing better comparisons between values.

Compounds **98** (glycine coupled) and **100** (valine coupled) resulted only between 1.3 to 1.5 times better binding monosaccharide mimics than methyl β-D-galactopyranoside. Valine may cause steric hindrance problems and glycine possesses a much more flexible chain than alanine. These two factors may prevent an improved fit into the binding pocket of H1-CRD. Similar relationships between binding affinities and the types of *N*-2 linked chain were reported in the literature by Iobst and Drickamer[32]. Binding of a series of *N*-acyl derivatives of galactosamine suggested a better interaction with the rat hepatic lectin than with Gal or even Gal*N*Ac. The highest binding affinity was observed for the *N*-propionyl derivative, similarly to the tendencies observed in our case (*table 22*). Among our compounds the alanine derivative **99**, which possesses a 3 C *N*-acyl chain coupled at the 2-position, also resulted in the best binder. Furthermore, the relative potency to Gal*N*Ac found for **99** is very similar to that one found in the literature for the *N*-iso-butanoylgalactosamine, which conserves a higher structural similarity with our alanine coupled derivative.

Table 22: Relative potencies to Gal*N*Ac of a panel of *N*-acyl derivatives. First, derivatives reported in the literature[32] tested towards the rat hepatic lectin are presented. Second, the monosaccharide mimics modified in the present work with amino acids at the *N*-2 position and tested towards H1-CRD are shown.

N-acyl derivatives[32]				
$\frac{IC_{50}}{IC_{50GalNAc}}$ (rHL-1)[a]	13 ± 1	0.13 ± 0.04	0.82 ± 0.06	0.29 ± 0.06

	 **98**	 **99**	 **100**
$\frac{IC_{50}}{IC_{50GalNAc}}$ (H1-CRD)	5	0.6	5

a) Iobst and Drickamer[32] informed the values as K_i. However, a competitive binding assay was performed and they defined K_i values as the concentration of monosaccharide giving 50% of inhibition. As a consequence, their values were employed as IC_{50} values for this comparative table.

Influence on the binding affinity of the combined actions of the 2- and 6-positions of galactose mimics.

The aim of the present chapter is the study of the combined influence and the impact on the binding affinity of substitutions at the 2- and 6-positions of the *galacto* moiety. It is of high interest to determine if an added effect could be observed and thus, the affinity for H1-CRD further optimized. Comparisons of the 2,6-disubstituted compounds with their parent mono substituted models will permit to evaluate the real contribution of each partner to the overall binding affinity.

The new combined strategy will take advantage of some of the benefits of solid phase chemistry. Reactions that were employed with satisfactory to very good yields can be now driven to completion by using excess of reagents and repeated reaction cycles.[45,54,99] The general approach in this thesis was the synthesis of various small libraries that will provide more diverse information than a single big library.[49]

A set of ten new compounds was synthesized by modifying the 2- and 6-positions of building block **91** (methyl 6-azido-2-deoxy-2-phthalimido-β-D-galactopyranose), immobilized on solid phase as **93**. This scaffold was developed in section **6.3.1** for amino acid couplings on solid phase. Firstly, amino acid couplings were performed as described in section **6.3.3**. Secondly, copper-(I)-catalyzed 1,3-dipolar cycloadditions between the 6-azido group and acetylenes were performed.

1.23 Solid phase preliminary experiments.

Solid phase reactions are not always as easily monitored as solution chemistry. For this reason a series of experiments were performed to reduce the number of possible problems along the synthetic pathway. Single problems were solved in advance and therefore avoided on the more complex system.

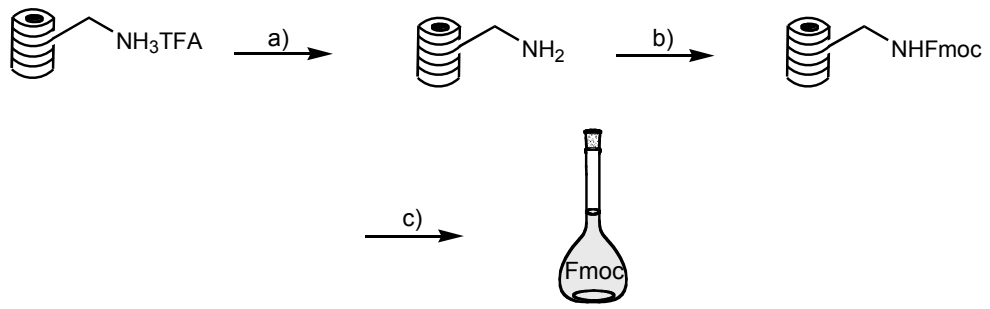
Experiments to optimize the solid phase procedure were run along the whole project. As a consequence, many of those tests were already presented. In this chapter, only a compilation of the performed studies can be found.

Loading capacity.

The aminomethylated polystyrene lanterns (Mimotopes SynPhase^{TM}, D series) used as polymer support possess a declared average loading capacity of 35 μmol per unit. This capacity was verified by our own methods to adjust the amount of starting material required and to exclude problems during loading and cleavage optimization. The method employed was based on the quantitative determination of an UV active specie loaded and subsequently cleaved from the support material.

The polymer support, provided in its trifluoro acetate form, was first let to swell in DMF followed by activation with triethylamine. After standard washing protocols, the polymer support was coupled with Fmoc-protected succinimidyl carbonate. Three hours later, the ninhydrin test[94] for detection of primary amines was negative (yellow color), indicating that the coupling was complete. The Fmoc group was then cleaved in a volumetric flask (*scheme 23*). The resulting solution, after adequate dilutions, was used for determining the Fmoc concentration, calculated from the UV absorption at 300nm and directly related with the amount of reacting sites in the lantern.

The average loading capacity determined was 37 μmol and was obtained from two measurements.



Scheme 23: a) i) DMF, r.t., 30 min, ii) 20% Et_3N in DMF, r.t., 3 x 10 min; b) 9-fuorenylmethyl *N*-succinimidyl carbonate, DIPEA, THF, r.t., 3 h;[100] c) 20% Piperidine in DMF, r.t., 30 min.

Linker stability assays.

The stability and compatibility with the chemistry of choice of the acetal linkage between compounds **1** or **2** and the various scaffolds employed, is summarized here.

The linker proved to be stable under different conditions for amide bond formation as well as Fmoc deprotection (sections **6.3.2** and **6.3.3**). Moreover, it also showed good stability under the click chemistry conditions (section **5.3.2**). In addition, it remained stable in presence of large excess of diethylamine, tetrabutylammonium fluoride, sodium methoxide and 1,5-diazobicyclo [4.3.0] non-5ene (DBN) (section **5.1.2**).

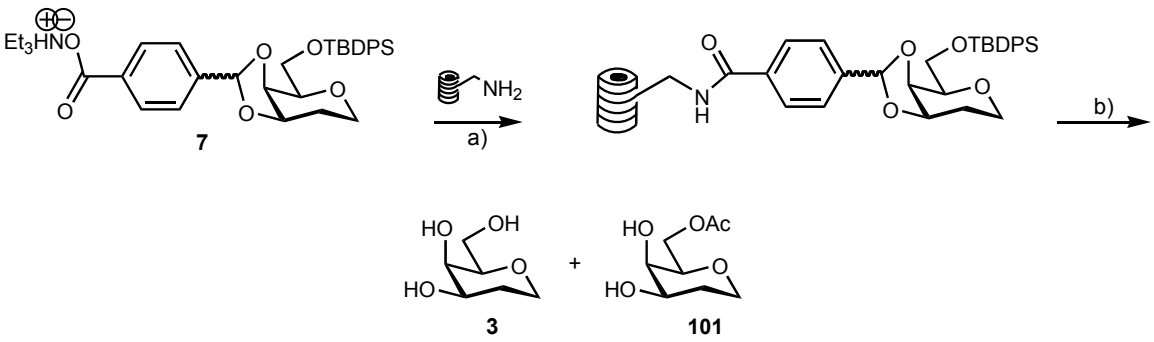
The versatility of the linker and linkage of choice was demonstrated and provides open access to many possible chemistry methods.

Loading and cleaving procedures.

Coupling the activated aminomethylated solid support with two different initial materials immediately followed by their cleavage from the solid phase helped to optimize both processes.

Compounds **7** and **93** (see also section **6.3.2**) were coupled with the solid phase using DIC and HOBt as activating reagents.[75]

Acid hydrolysis of the acetal connection between linker and scaffold was employed for cleaving the immobilized material. Treatment of the lantern with 80% aqueous AcOH containing 2% of trifluoroacetic acid (TFA)[55] was compared with milder methods such as 4% TFA in MeOH or dioxane.[57] These two last methodologies did not provide the expected cleaved material. Nevertheless, the first method provided the expected compounds in satisfactory yields. In the case of compound **7**, a mixture of products was obtained after cleavage caused by the deprotection of the acid labile TBDPS group. The fully deprotected hexitol **3** and its 6-acetylated derivative (**101**) were obtained. The recovery represented 75 % of the theoretical loading capacity (*scheme 24*).



Scheme 24: a) i) 20% Et_3N, DMF, r.t. 3 x 10 min, ii) HOBt, DIC, DMF, r.t., 15 h;[75] b) 80% aqueous AcOH, 2% TFA, r.t., 2 x 15-20 h.[55]

1.24 Synthesis of the library on solid phase.

A collection of 10 derivatives (**102** to **111**) of methyl β-D-galactosamine, modified at the 2- and 6-positions on solid support, was synthesized.

Scaffold **91** (*scheme 19*) was coupled with **2** and immobilized on the polystyrene lanterns. First, following the removal of the phthalimido protection at the 2-position, amino acid couplings were performed. The same procedure used in section **6** was employed. Five consecutive HBTU mediated standard coupling cycles in the presence of HOBt and DIPEA were applied to drive the reactions to completion at the hindered 2-amino group. Glycine, alanine and valine were the selected amino acids. Second, the azido group introduced at the 6-position of the sugar moiety was employed for performing copper-(I)-catalyzed 1,3-dipolar cycloadditions between it and a number of acetylenes. The successful trial for click chemistry on solid phase by immobilization of **49** (section **5.3.2**), indicated a good transposition from solution to solid phase of this type of chemistry.

Click chemistry optimization.

Performing 1,3-dipolar cycloadditions on the new immobilized scaffold **91** (or **93**) involved many optimization efforts to obtain quantitative single products. In spite of the lower reactivity of the methyl 2-*N*-acyl-2-deoxy-6-azido-β-D-galactoside mimic for 1,3-dipolar cycloadditions than its hexitol counterpart it was possible to optimize conditions in order to obtain quantitative conversions. In all the cases tested, excess of acetylenes was employed and reactions were run during four consecutive days, starting each day a new synthetic cycle. Each cycle was started by treatment of the lantern with a freshly prepared solution of the corresponding alkyne. Four to five additions of sodium ascorbate and copper sulfate as to produce *in situ* the catalyst, were performed every 3 hours during the course of one cycle.

In all the attempts during optimization, the recoveries of loaded material were satisfactory (*table 23*), indicating good performance of every other step involved in the methodology. For the calculations of the recovered material, the MWs of the corresponding acetates were used considering that after cleavage the acetate form of the derivatives modified by amino acid coupling was obtained.

The protocol used for modifying the hexitol derivative on solid phase by click chemistry (section **5.3.2**), was initially applied on the polymer bound **93**. This method involved three consecutive cycles of reaction (one day per cycle) and three equivalents of acetylene each time. However, the expected product could not be obtained under these conditions for the new scaffold **93** (entry 1, *table 23*). As a consequence, different experiments were run to determine the optimal conditions (*table 23*).

First, the amount of alkynes employed was increased to 9 and 10 equivalents. However, even if a slight improvement could be observed, results were still not satisfactory as to obtain a robust methodology. Employing 9 equivalents of 3-ethynyl thiophene (entry 2, *table 23*) generated a mixture of two products in the cleaved material. Only 43% was the expected product and the rest was the 2-*N*-acyl modified compound synthesized in the previous step (amino acid coupling). However, for other alkynes either no reaction was observed or in the case of 3-ethynyl-α,α,α-

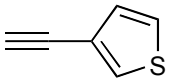
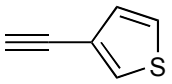
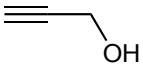
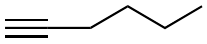
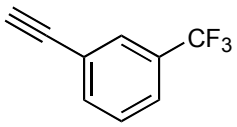
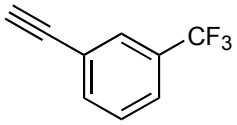
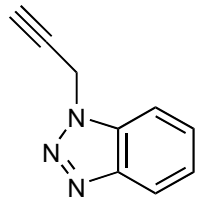
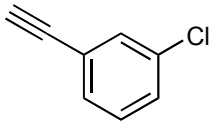
trifluorotoluene (entry 5, *table 23*) only traces of the product were detected. The product cleaved from solid phase resulted to be compound **99**.

As a consequence of the problems found during optimization on solid phase, studies in solution were performed to investigate about the intrinsic reactivity of the scaffold without the influence of the solid phase that renders the reactive sites less accessible.

Compound **99**, was then reacted in solution for obtaining the [1,2,3]-triazole. After two days of reaction and having added in total 15 equivalents of the corresponding alkyne (3-ethynyl-α,α,α-trifluorotoluene), only a very small amount of the expected product was obtained (entry 6, *table 23*). Two products could clearly be detected in the crude mixture obtained after the reaction. The presence of the triazole in one of them was clear due to the strong shift to lower fields experienced for the H-6 protons in the ^{1}H NMR spectrum (H-6: **99** → **109** δ 3.24 and 3.68 ppm → 4.77 ppm). After purification of the material by reversed phase chromatography, with a gradient of 5% MeOH in H_2O, only 27% of the material recovered was the expected 1,4-disubstituted-[1,2,3]-triazole **109**, while the rest was compound **99**. This trial put into evidence two points: first it was shown that the azido group was intact and reactive; second, it emphasized the intrinsic lower reactivity of the 6-azido in the galactose mimic **91** compared with the hexitol derivative **46**. The observation justified an important increase of the equivalents of alkyne employed in solid phase considering on one hand the already high quantities required in solution and on the other hand, the excess usually demanded by solid phase reactions.

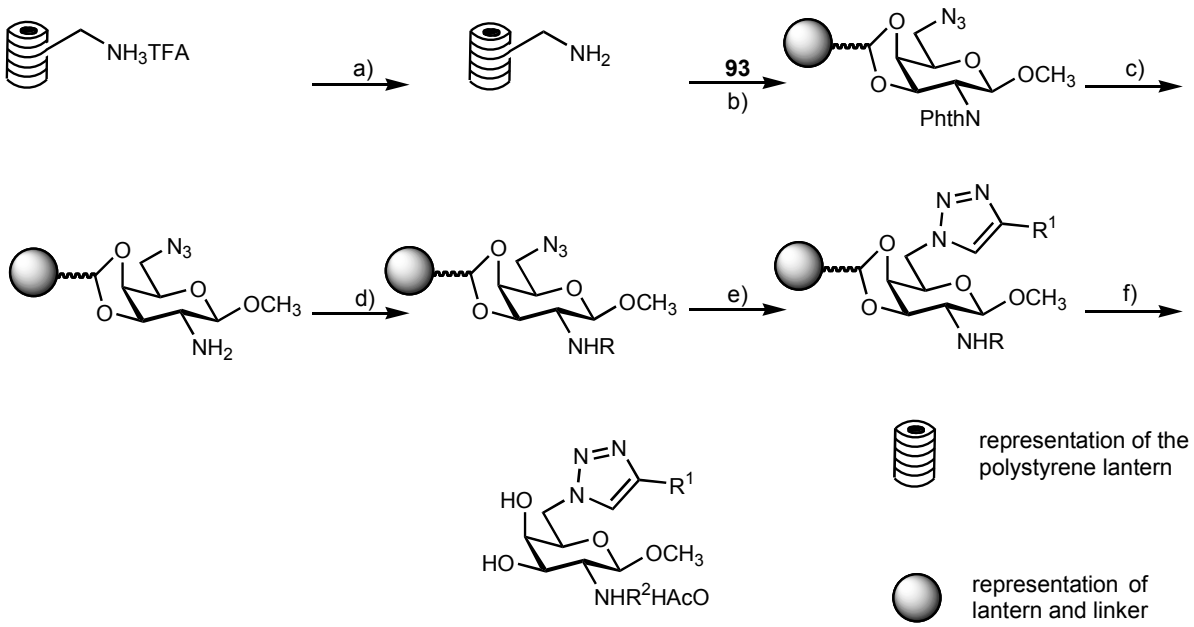
Finally, by increasing the amount of alkyne equivalents to 30, the expected triazoles could be afforded in quantitative yields independent of the acetylenes employed and with more than 98% purity according to ^{1}H NMR results. (*scheme 25*)

Table 23: Trials for solid phase optimization of 1,3-cycloaddition reactions on the immobilized scaffold **93**. Previous modifications by amino acid couplings at the 2-position were performed.

Entry	Alkyne	Equivalents per cycle	Type of chemistry[a)]	Outcome	Recovery
1		3	SP	Not obtained	79%
2		9	SP	43%	90%
3		10	SP	Not obtained	91%
4		10	SP	Not obtained	86%
5		10	SP	Not obtained	89%
6[b)]		15	Solution	27%	-
7		30	SP	quantitative	60%
8		30	SP	quantitative	80%

a) SP means “solid phase” reactions. b) Reaction performed in solution using as starting material compound **99** obtained from the cleaved material from entry 5.

The difficulties found in the triazole formation at the 6-position of **93** compared to the use of **49** as scaffold could be explained by the more electro deficient azido group of **93** consequence of the effect of the close anomeric center.



102-**111**

R = *N*-acyl-Fmoc protected from Gly, Ala or Val
R^1 = Substituent of the alkyne employed
R^2 = Fmoc deprotected *N*-acyl in its acetate form

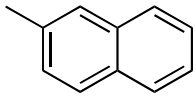
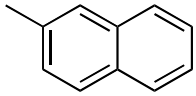
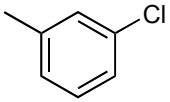
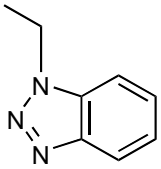
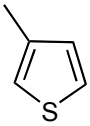
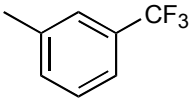
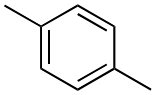
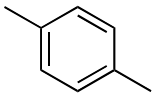
Scheme 25: a) i) DMF, r.t., 30 min, ii) 20% Et_3N, DMF, r.t., 3 x 10 min; b) DIC, HOBt, DMF, r.t., 15 h;[75] c) $H_2NNH_2 \cdot H_2O$, MeOH, 80°C, 4 x 3 h, 1 x 12 h;[98] d) Ala-Fmoc, HBTU, HOBt, DIPEA, DMF, r.t., 3 x 4 h, 2 x 12 h; e) Alkyne (30 eq), sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*-BuOH (1:1),[47] r.t., 4 x 24 h; f) i) 20% Piperidine, DMF, r.t., 3 x 10 min, ii) 80% aqueous AcOH, 2% TFA, r.t., 2 x 15-20 h.[55]

Synthesis of the library.

A collection of 10 methyl β-D-galactoside mimics (**102** to **111**), modified at the 2- and 6-positions on solid phase, was successfully synthesized (*table 24*).

During the library production all compounds that could not be previously obtained during the optimization process with fewer equivalents of alkynes were obtained in quantitative amounts with the new methodology developed. That is the case of compounds **104**, **108** and **109** (*table 23*).

Table 24: Compounds synthesized on solid phase modified at the 2- and 6-positions of building block **93**.

Compound	R^1	$R^{2\ a)}$	Recovery $^{b)}$
102		Gly	100%
103		Gly	96%
104		Ala	82%
105		Ala	93%
106		Ala	80%
107		Ala	60%
108		Ala	100%
109		Ala	93%
110		Ala	100%
111		Val	100%

R^1 and R^2 as in *scheme 23*. a) *N*-acyl derivative from Gly, Ala or Val. After cleavage, the acetate form was obtained. After reversed phase chromatography the free amino group was afforded. b) Percentage of loaded material recovered after cleavage considering 35 μmol as the theoretical capacity of one lantern.

^{1}H NMR spectroscopy was performed with the materials obtained after cleavage and evaporation until dryness with a geenevac. For all new entities except **103** and **104**, only one product could be detected with purities equal or above 98%. In the case of

102 and **104**, the crude ^{1}H NMR was very affected by rests of copper, not allowing the direct identification of the products. However the signal corresponding to the H-6 protons was clearly identified. The strong shift suffered by the H-6 signal to lower fields indicated the formation of the triazole ring. In addition, only the mass of the expected product could be found in mass spectrometry after sample filtration. As a consequence, it is possible to affirm that the desired products were obtained in these two cases as well but it was impossible to determine their purity directly after cleavage. The free amino group of the compounds was only obtained after purification by reversed phase chromatography.

Purification.

All compounds obtained from solid phase synthesis were purified before submitting them to the competitive target-based assay. Different methods for purification by LC-MS were tried.

First, an analytical run for compound **107** using a SunFire™ C18 column with CH_3CN/H_2O with additional 0.2% formic acid as mobile phase was performed. The linear gradient run from 5% to 95% acetonitrile in 7 min followed by 2 min at 95%, before returning to the initial conditions in another 2 min. The product came with the injection peak and no separation at all was achieved. Second, the system was run free of formic acid to avoid the protonation of the amino acid and the triazole rings present. However, no modification of the elution pattern was observed.

Third, a change in the elution gradient from the standard one was tried. In absence of formic acid, 100% water was used as mobile phase and only after the first 8 min a 1:1 mixture of CH_3CN/H_2O was applied for 2 min to return then to the initial solvent. Once again the peak corresponding to the expected mass was found with the injection one.

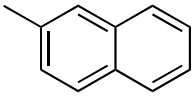
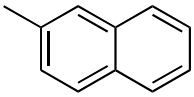
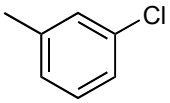
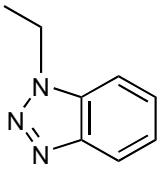
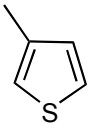
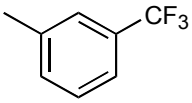
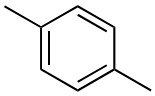
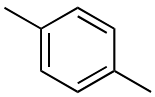
Finally, a new type of silica gel based column that allows better interactions with basic substances was employed. A Reprosil-Pur basic analytical column from Dr. Maisch GmbH (5 µm, 4.6 x 250 mm) was employed with a flow of 1 mL/min and was submitted to a linear gradient of CH_3CN/H_2O as in the standard procedure with absence of formic acid. In this case, a good separation was achieved. The retention time of the peak of interest was 8.31 min. The method could therefore be employed

for purifying the library in an automated way. However, a preparative column of these characteristics was not available in our facilities. As a consequence, each compound was manually purified by reversed phase chromatography with a 5% MeOH gradient in H_2O.

1.25 Competitive target-based assay (In collaboration with D. Stokmaier and Dr D. Ricklin from the Institute of Molecular Pharmacy, University of Basel).

Methyl β-D-galactoside mimics **102** to **111** were submitted to the competitive target-based assay for determination of their IC_{50} values (*table 25*).

Table 25: IC_{50} (μM) values determined by competitive target-based assay for compounds **103**-**112**.

Compound	R^1	$R^{2\ a)}$	IC_{50} (μM)
102		Gly	228 ± 176
103		Gly	403 [b)]
104		Ala	98 ± 2
105		Ala	34 ± 4
106		Ala	126 ± 29
107		Ala	64 ± 46
108		Ala	63 ± 38
109		Ala	48 ± 15
110		Ala	62 ± 0.4
111		Val	287 [b)]

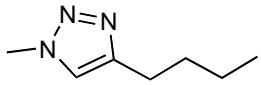
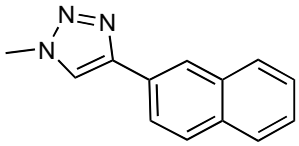
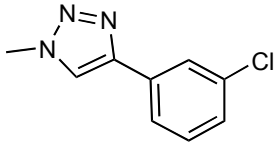
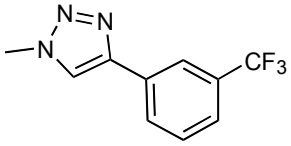
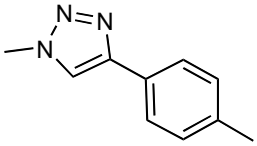
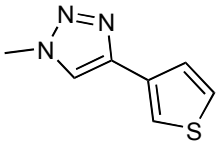
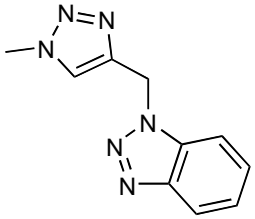
R^1 and R^2 as in **scheme 23**. *a) N*-acyl derivative from Gly, Ala or Val. Values are the media ± standard deviation of two independent measurements except for b) where only one measurement was done.

Good reproducibility between independent determinations was in general obtained. Firstly, all compounds had improved binding affinities towards H1-CRD relative to methyl β-D-galactopyranoside. All derivatives coupled with alanine had lower IC_{50} values than their glycine or valine counterparts. The same general behavior observed for the compounds modified only at the *N*-2-position with *N*-acyl chains (**98** to **100**)

was observed. That is to say, 2,6-disubstituted compounds with alanine coupled at the *N*-2 were all in the binding affinity range of Ga*l*N*N*Ac and in most of the cases even slightly lower. For all compounds coupled with one specific amino acid no preference between the various substituent types of the triazoles could be observed. Moreover, no changes in the binding affinity were experienced as a consequence of the different modifications introduced at the 6-position. (*table 26*)

Only the triazole ring might be able to establish contact with the Trp 243 of the receptor. Nevertheless, the 4-substituents at the triazole do not interfere with the binding and bulky substituents could be accommodated. The 6-position of the studied derivatives does not further improve the binding affinity of the 2-substituted compound but it also does not interfere with it.

Table 26: Comparison of IC_{50} (μM) values for 2,6-substituted galactose mimics and their mono substituted parents.

R [a]	Gly [b]	Ala [b]	Val [b]	Hexitol mimics [c]
N_3	401	46 ± 9	385 ± 61	248 ± 41
	228 ± 176	98 ± 2	-	-
	403 [b]	34 ± 4	-	Precipitated during assay
	-	126 ± 29	-	-
	-	48 ± 15	-	481 ± 127
	-	62 ± 0.4	287 [b]	-
	-	63 ± 38	-	421 [d]
	-	64 ± 46	-	-

a) R is the 6 substituent. b) *N*-acyl derivative from Gly, Ala or Val. c) Hexitol mimics **47**-**49**. Values are the media ± standard deviation of two independent measurements except for d) where only one measurement was performed.

Conclusion and outlook

Summary of the thesis.

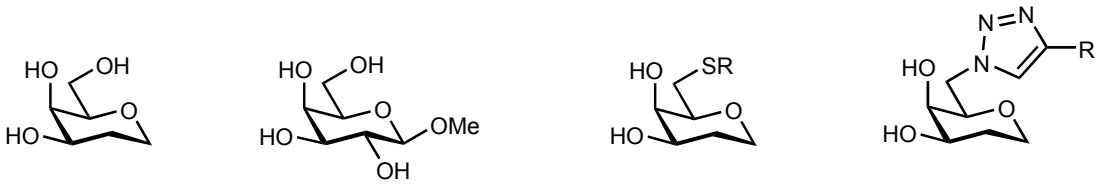
The ASGP-R, exclusively located on the cell surfaces of hepatocytes, is well known for removing desialylated glycoproteins with terminal galactose or *N*-acetyl*g*alactosamine residues from circulation. However, small high affinity ligands with more drug-like properties than the natural glycoproteins, that can be used for specific targeting of the liver, were not yet investigated for the human H1-CRD. Various small-directed libraries of galactose derivatives and mimics thereof were synthesized in this work. The design of the ligands was based on the crystal structure of the H1-CRD[10], and on data from *in silico* studies[40] as well as from previous SAR studies for similar receptors. The influence of different types of modifications at the *galacto* moiety on the binding affinity, were analyzed by a competitive target-based assay and Biacore experiments, both performed with immobilized H1-CRD. Monosaccharide mimics with slightly improved binding affinities for the H1-CRD relative to Gal*N*Ac were found. This work provides experimental data for simple synthetic ligands of the H1-CRD and therefore gives guidelines for the rational design of a novel generation of improved ligands.

1.26 Galactose mimics modified at their 6-position.

Galactose mimics based on the 1,5-anhydro-2-deoxy-D-*lyxo*-hexitol core were modified at their 6-position by conjugated additions, nucleophilic substitutions and 1,3-dipolar cycloadditions. Compound **3** was used as scaffold (*figure 14*). By substituting its 6-position it was attempted to establish hydrophobic interactions with the Trp 243. Due to the reduced number of sites with similar reactivity, short synthetic pathways provided fast access to these ligands.

All the derivatives competed approximately two to three-fold better for H1-CRD than Gal (*figure 21*). Binding affinities comparable to the one of methyl β-D-galactopyranoside were obtained. Since for derivatives modified with 1,4-disubstituted-[1,2,3]-triazoles (**45**, **50**), the behavior was independent of the 4-

substituent at the triazole, the interaction with Trp 243 is possibly restricted to the triazole ring. The 6-position is directed towards the surrounding water and bulky substituents could be accommodated without interfering with the binding.



3
IC_{50} (μM) 1399± 368
K_D = 1480

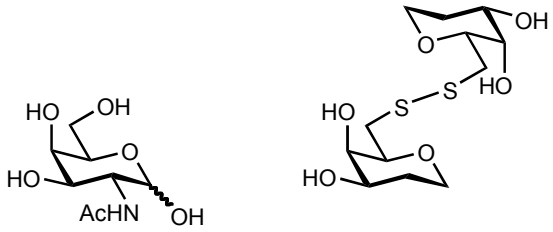
Methyl β-D-Gal
IC_{50} (μM) 545 ± 39
K_D = 1780

28-**38**
IC_{50} (μM) 690 to 310

45, **50**
IC_{50} (μM) 610 to 420

Figure 21: Reference compound **3** and general structures of galactose mimics synthesized. Minimum and maximum IC_{50} (μM) values, considering the standard deviations, are indicated for each family of compounds. Two independent determinations were performed, except for **3** where three independent measurements were done.

Compound **39**, obtained as secondary product from Michael additions and nucleophilic substitutions, showed a comparable binding affinity as Gal*N*Ac (f*igure 22*) in the competitive target-based assay. However, Biacore monitoring of the direct interaction between **39** and the immobilized H1-CRD did not show a comparable increase of the binding affinity. Since hexitol **39** has two pairs of free 3,4-hydroxyls in equatorial and axial arrangement, the difference may come from a local concentration effect which could convert two weak binders into one highly improved binder.[34,36,45]



Gal*N*Ac
IC_{50} (μM) 78± 5

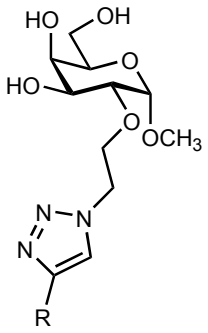
39
IC_{50} (μM) 94 ± 18

Figure 22: IC_{50} (μM) values obtained from the competitive target-based assay. The values are the media ± standard deviation of two independent determinations.

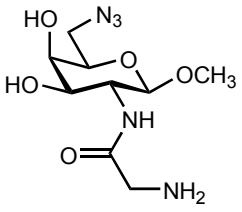
In general, galactose mimics modified at the 6-position by hydrophobic groups showed only a modest improvement of the binding affinity for H1-CRD relative to Gal. However, bulky groups in the 6-position did not interfere with the binding indicating that this position can be used to link the targeting device with a possible drug.

1.27 Galactose derivatives and mimics thereof modified only at their 2-position.

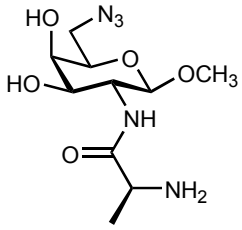
To investigate the role that modifications at the 2-position of the galactose moiety may play, two families of derivatives were synthesized (*figure 23).*



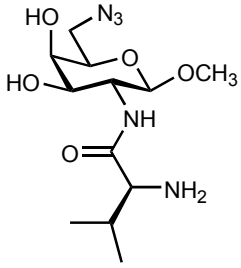
66 to **75**
IC_{50} (μM) 592 to 1209



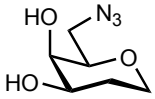
98
IC_{50} (μM) 401
K_D (μM) 1120



99
IC_{50} (μM) 71± 32
K_D (μM) 134



100
IC_{50} (μM) 385± 61
K_D (μM) 1268



46
IC_{50} (μM) 248± 41
K_D (μM) 855

Figure 23: Two families of galactose derivatives modified at the 2-position and galactose mimic **46**.

Compounds **66** to **75** were obtained by 1,3-dipolar cycloadditions performed on an azido group at the C-2' linked to the 2-OH of the sugar scaffold. They do not show

improved binding affinity in the competitive assay and the Biacore experiments. Derivative **72** was the only member of this group to show a binding affinity (IC_{50} (μM) = 110 ± 13) comparable to the one of Gal*N*Ac. However, a different binding mode was found by biosensor studies of the direct interaction between this compound and the immobilized monomer of H1-CRD.

2-*N*-acyl derivatives of a methyl 6-azido-β-D-galactopyranoside were synthesized on solid phase by amino acid coupling at the *N*-2-position of the sugar moiety. Compounds **98**-**100** showed improved binding affinities for the H1-CRD relative to methyl β-D-galactopyranoside (*figure 23*). Whereas derivatives acylated with glycine (**98**) or valine (**100**) experienced only a modest improvement of their binding affinities, the 2-(2-amino propionamido) derivative **99** achieved a binding affinity approximately 1.5 to 2-fold better than Gal*N*Ac and 12-fold better than methyl β-D-galactopyranoside. Even though the galactose mimic **46** containing an azido functionality at the 6-position, competed 2 to 3 times better for binding to the H1-CRD relative to **3**, the 6-azido group present also in derivatives **98**-**100** does not contribute to the binding.

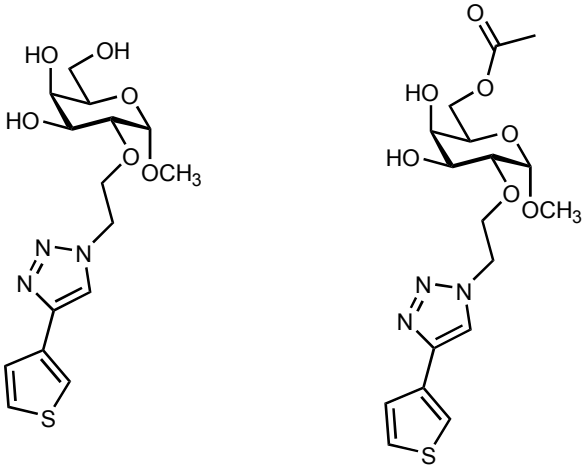
N-acyl coupled chains appear to improve the binding affinity for the receptor depending on the size of the acyl group. The propionamido derivative displayed the lowest IC_{50} value and therefore the optimal chain length studied. It is possible that such *N*-acyl derivatives allow establishing an optimal interaction with His 256, through H-bond formation with the hydrogen of the nitrogen of the *N*-acyl group. This interaction is known for being responsible for the preferential binding of Gal*N*Ac with respect to Gal. 2-*N*-acyl derivatives are an excellent starting point for the development of novel high affinity ligands for the H1-CRD.

1.28 Monosaccharides mimics modified simultaneously at their 2- and 6-positions.

Methyl β-D-galactosamine-derivatives modified at the 2- and 6-positions were synthesized to elucidate the hypothesis of an added effect on the binding affinity. A collection of ten di-substituted galactose mimics was synthesized on solid phase with

excellent yields. Amino acids were first coupled with the *N*-2-position of a galactosamine mimic and modified Huisgen 1,3-dipolar cycloadditions were performed at the 6-position of the monosaccharide mimic.

Results from the competitive target-based assay indicated that all the derivatives synthesized were better binders for the H1-CRD than methyl β-D-galactopyranoside. However, all di-substituted compounds showed very similar IC_{50} values to their parent derivatives *N*-acyl monosubstituted (**98**-**100**). Improved binding due to the additional 6-substitution could not be observed. The better-defined orientation of the monosaccharide in the binding pocket, after establishing additional interactions through its 2-*N*-substituent, could be the reason for the loss of a complementary 6-interaction. In addition, this hypothesis may also explain the behavior observed in the preliminary IC_{50} determination for compound **69** relative to **68** (*figure 24*). In the presence of the substitution in 2-position, compound **68** did not showed an improved binding affinity compared to Gal. Lacking good interactions with the protein in the region of the 2-position of Gal, the substituent at the 6-position in **69** could improve binding.



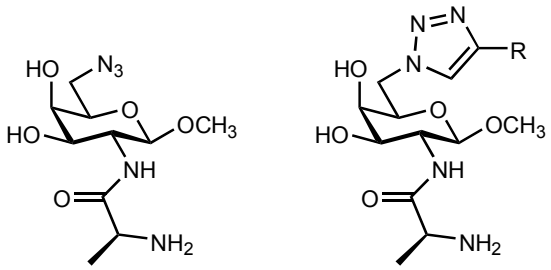
68
IC_{50} (μM) 952

69
IC_{50} (μM) 592

Figure 24: Influence of the 6 position in a weak binder.

Since none of the substituents employed at the 6-position interfered with the binding, a collection of eight derivatives obtained by amino acid coupling with alanine (**99** and

104-**110**), with similar or slightly improved binding affinities than Gal*N*Ac was obtained for the H1-CRD (*figure 25*).



99
IC_{50} (μM) 71 ± 32

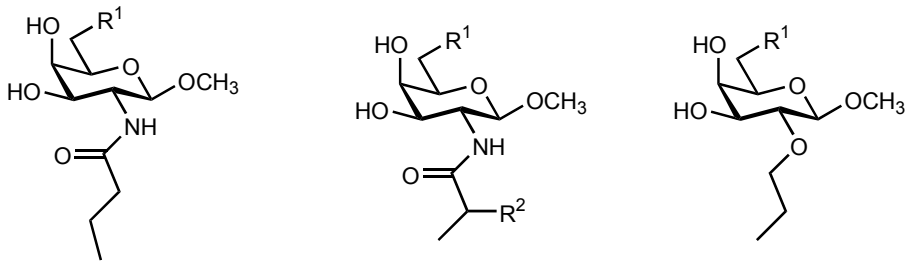
104 to **110**
IC_{50} (μM) 155 to 30

Figure 25: High affinity galactose mimics synthesized for the H1-CRD.

Outlook.

The results obtained in this thesis may be applied to produce a new generation of ligands for the H1-CRD.

First, the synthesis of three 2-substituted galactose derivatives (*figure 26*) may be envisaged.



112 **113** **114**

Figure 26: Further exploration of the binding site; R^1: OH, N_3; R^2: H, CH_3, OH.

The influence of the one-carbon extension of the *N*-acyl chain of **99** on the binding affinity could be studied with compound **112**. Between the propionamido derivative **99** and the valine-derivatized compound **100** exists a space to be analyzed for determining the real optimal length preferred for the *N*-acyl chain. Moreover, the absence or replacement of the α-amino group on the *N*-acyl chains (**113**) would allow further exploration of the binding site and the type of established interactions. Substituents as H, CH_3 and OH are suggested in order to determine whether hydrophobic interactions or H-bond formation are preferred. Additionally, compound **114** will help to establish if the *N*-acyl derivatives have a preferred binding relative to their *O*-counterparts due to specific interactions with His 256.

Second, compound **99** could be employed to synthesize a triantennary ligand[36] for the H1-CRD. Determination of the IC_{50} value of the novel multivalent ligand in a competitive assay and comparison with a triantennary galactose ligand would be of high interest for the development of liver targeting strategies. Additionally, a comparative study of its cellular uptake by the hepatocytes would also be required.

Experimental

General

NMR spectra were recorded on a Bruker Avance DMX-500 (500 MHz) spectrometer. Assignments of ^{1}H and ^{13}C NMR spectra were achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD and HDO as references. Optical rotations were measured using Perkin-Elmer Polarimeters 241 and 341. ESI-MS and LC-MS analyses were carried out with a Waters micromass ZQ spectrometer equipped with ELS detector (waters 2420) and photodiode array detector (waters 2996). All spectra were recorded in positive mode. The LC-MS was equipped with an analytical SunFire^{TM} column from Waters C18 (3.5 μm, 2.1 x 50 mm) and 0.2 mL volume. The mobile phase was CH_3CN/H_2O with additional 0.2% formic acid, using a flow rate of 0.5mL/min. The linear gradient run from 5% to 95% acetonitrile in 7 min followed by 2 min at 95% before returning to the initial conditions in another 2 min. IR spectra were obtained with a Perkin-Elmer Spectrum One FT-IR spectrometer. Elemental analyses were carried out with a Perkin-Elmer 240 analyzer. Reactions were monitored by TLC using glass plates coated with silica gel 60 F_{254} (Merck) and visualized by using UV light and/or a developing solution (0.02M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in 10% H_2SO_4). Column chromatography was performed on silica gel (Fluka 60). Reversed- phase chromatography was done on LiChroprep RP 18 (Merck). Methanol (MeOH) was dried by refluxing with sodium methoxide. Pyridine (Py) was freshly distilled over CaH_2. Tetrahydrofuran (THF) was dried by refluxing with sodium and benzophenone. *N,N'*-dimethylformamide (DMF) was distilled under vacuum and immediately stored over activated molecular sieves 4 Å. Acetonitrile (CH_3CN), dichloroethane (DCE), dichloromethane (DCM), dioxane and hexane were dried by filtration over Al_2O_3 (Fluka, Brockmann activity I). Petroleumether (PE) was distilled over $CaCl_2$. Ethyl acetate and *tert*-butanol were used without further purification. Molecular sieves type 3 Å and 4 Å were activated in vacuum at 600°C immediately before use. As support for the solid phase synthesis, aminomethylated polystyrene lanterns (Mimotopes SynPhase, D series) were employed. The average loading of each lantern was 35 μmol.

Ninhydrin test for primary amines.

A washed piece of lantern is treated with 6 drops of solution A and 2 drops of solution B and heated for 10 min at 100°C. Blue staining indicates the presence of primary amines.

Solution A: Solution 1: Phenol (40 g) is dissolved in abs. EtOH (10 mL), stirred with Amberlite MB-3 mixed bed resin (4 g) for 45 min and filtered. Solution 2: KCN (1.3 g) is dissolved in H_2O (2 mL) and diluted with pyridine (100 mL, freshly distilled from ninhydrin). The solution is stirred over Amberlite MB-3 mixed bed resin (4 g) for 45 min and filtered. Solutions 1 and 2 are mixed to obtain solution A.

Solution B: Ninhydrin (2.5 g) dissolved in abs. EtOH (50 mL).

Determination of the loading capacity.

An aminomethylated lantern (Mimotopes SynPhase^{TM}, D series) was left for 15 min in DMF and then treated with 20% Et_3N in DMF (3 x 10 min) for activation. The lantern was then washed with DMF (3 x 10 min) and DCM (3 x 10 min). A suspension of Fmoc-succinimidyl carbonate (150 mg, 0.445 mmol) in dry THF (540 μL) was prepared. 457 μL (0.376 mmol of Fmoc-succinimidyl carbonate) of the solution and DIPEA (43 μL, 0.25 mmol) were combined in a well of a 96-well plate. The lantern was added and the plate was left in the shaker at r.t. After 3 h the ninhydrin test[94] for primary amines was negative. The lantern was washed with THF (2 x 10 min), DMF (2 x 10 min) and DCM (2 x 10 min). The lantern was added to a 10 mL volumetric flask filled with 20% piperidine in DMF and left in the shaker at r.t. for 30 min. 0.5 mL of the resulting solution were diluted in a second volumetric flask (10 mL) with 20% piperidine in DMF. An UV scan between 190 nm and 400 nm was performed with a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer. The absorption at 300 nm of two measurements was used for determination of the loading capacity with the following formula:

$$\text{Loading } (\mu\text{mol}) = \frac{200 \bullet A_{300nm}}{7.8}$$

A_1 = 1.428;

A_2 = 1.459.

Loading capacity: 37 ± 0.6 μmol.

Synthesis of the linkers

Allyl 4-formyl-benzoate (1), (CR 1).

Cesium fluoride-celite was formed by adding CsF (5.04 g, 33.2 mmol) to a suspension of celite (3.01 g) in H_2O (100 mL). After stirring at r.t. for 40 min, the solvent was evaporated under reduced pressure and the solid residue washed with CH_3CN (30 mL). 4-Dimethoxymethyl-benzoic acid (2.17 g, 11.1 mmol), was dissolved under argon in CH_3CN (300 mL). Dry CsF-celite (2.52 g of CsF, 16.6 mmol) was added to the solution followed by allyl bromide (1.92 mL, 22.2 mmol). The mixture was refluxed at 95°C for 20 h. After cooling to r.t., the solvent was evaporated under reduced pressure, the residue was taken up in EtOAc (20 mL), filtered and the filtrate concentrated. Purification was done by silica gel chromatography (PE/EA 6:1, +1% Et_3N) to obtain **1** (1.94 g, 93%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.85 (m, 2H, $CH_2CHC\mathbf{H}_2O$), 5.32 (d, *J* = 10.4 Hz, 1H, Ha of $C\mathbf{H}_2CHCH_2O$), 5.42 (d, *J* = 17.2 Hz, 1H, Hb of $C\mathbf{H}_2CHCH_2O$), 6.04 (m, 1H, $CH_2C\mathbf{H}CH_2O$), 7.95, 8.21 (m, 4H, C_6H_4), 10.10 (s, 1H, COH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 66.26 ($CH_2CH\mathbf{C}H_2O$), 118.95 ($\mathbf{C}H_2CHCH_2O$), 129.65, 130.37, 131.91, 135.25, 139.32 (7C, $CH_2\mathbf{C}HCH_2O$, C_6H_4), 191.74 (COH).

Allyl 4-dimethoxymethyl-benzoate (2), (CR 2).

Compound **1** (576 mg, 3.03 mmol) was dissolved in MeOH (30 mL) under argon. Trimethyl orthoformate was added to the solution. A catalytic amount of *p*-toluene sulfonic acid monohydrate (57.6 mg/mL in 0.50 mL MeOH) was added and stirring continued for 30 min. The reaction was quenched by neutralization with Et_3N (10 mL) and the solvents were evaporated under reduced pressure. Purification by silica gel chromatography (PE/EA 8:1, +1% Et_3N) yielded **2** (698 mg, 97%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.33 (s, 6H, $2OCH_3$), 4.83 (m, 2H, $CH_2CHC\mathbf{H}_2O$), 5.30 (m, 1H, Ha of $C\mathbf{H}_2CHCH_2O$), 5.43 (m, 2H, Hb of $C\mathbf{H}_2CHCH_2O$, $C\mathbf{H}(OCH_3)_2$), 6.05 (m, 1H, $CH_2C\mathbf{H}CH_2O$), 7.54, 8.07 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 53.05 (OCH_3), 66.00 ($CH_2CH\mathbf{C}H_2O$), 102.73 ($\mathbf{C}H(OCH_3)_2$), 118.70 ($\mathbf{C}H_2CHCH_2O$), 127.22, 130.00, 132.60, 143.41 (7C, $CH_2\mathbf{C}HCH_2O$, C_6H_4); MS (ESI): Calcd for $C_{13}H_{16}O_4$ M^+: 236.10; Found m/z: 236.03; IR (NaCl) ν 1722 vs (CO) (cm^{-1}).

Synthesis of hexitols as mimics of galactose for modifications at the 6-position

3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*lyxo*-hexitol (4), (CRIV 1).

3,4,6-Tri-*O*-acetyl-D-galactal (5.45 g, 20.0 mmol) was dissolved in MeOH (20 mL) under argon. Palladium on activated charcoal (10%, 250 mg) was added. The mixture was stirred vigorously under an atmosphere of H_2 at r.t. for 15 h. The reaction was diluted with DCM (30 mL) under argon and then filtered through a pad of celite. The filtrate was concentrated under reduced pressure to obtain **4** (5.40 g, 99%) as colourless oil. The product was used in the following step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.69 (m, 1H, H-2a), 2.01-2.15 (m, 10H, $3CH_3$, H-2b), 3.58 (t, $J_{1a,1b}$ = 12.2 Hz, 1H, H-1a), 3.75 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 1H, H-5), 4.06-4.14 (m, 3H, H-6, H-1b), 4.99 (m, 1H, H-3), 5.30 (d, $J_{3,4}$ = 1.4 Hz, 1H, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.70, 20.84, 20.97 ($3CH_3$), 26.38 (C-2), 62.55 (C-6), 65.86 (C-1), 66.89 (C-4), 69.66 (C-3), 74.86 (C-5), 170.03, 170.27, 170.48 (3CO).

1,5-Anhydro-2-deoxy-D-*lyxo*-hexitol (3), (CRIV 2).

Compound **4** (5.40 g, 19.7 mmol) was dissolved in MeOH (120 mL) under argon. Sodium methoxide was added to the solution in small aliquots until the pH of the reaction was slightly basic (pH 8-9). Then the mixture was stirred at r.t. for 4h. The reaction was neutralized by addition of amberlyste 15 ion-exchange resin and filtered through a pad of celite. The mixture was concentrated under reduced pressure to give **3** (2.85 g, quantitative), which was used without further purification in the next step. ^{1}H NMR (500 MHz, CD_3OD): δ 1.62, 1.94 (m, 2H, H-2), 3.35 (m, 1H, H-5), 3.46 (m, 1H, H-1a), 3.62-3.77 (m, 4H, H-3, H-4, H-6), 3.98 (m, 1H, H-1b); ^{13}C NMR (125 MHz, CD_3OD): δ 30.46 (C-2), 63.84 (C-6), 67.42 (C-1), 70.16 (C-4), 71.15 (C-3), 81.29 (C-5); Anal. calcd for $C_6H_{12}O_4$: C 48.64; H 8.16; Found: C 48.86; H 8.07.

1,5-Anhydro-2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-D-*lyxo*-hexitol (5), (CRIV 3).

Dry hexitol **3** (2.92 g, 19.7 mmol) was suspended in DCM (60 mL) under argon. To this suspension *tert*-butyl diphenylchlorosilane (5.6 mL, 22 mmol), DMAP (96 mg, 0.79 mmol) and Et_3N (3.0 mL, 23 mmol) were added. The reaction was vigorously stirred at r.t. for 24 h. The reaction was driven to completion by repeated additions of TBDPS (total 2.0 mL, 7.9 mmol) and Et_3N (total 0.80 mL, 6.1 mmol). The solvent was

evaporated under reduced pressure and the residue was taken up in DCM (20 mL). The organic phase was washed with water (15 mL) and brine (2 x 15 mL). The combined organic layers were dried with Na_2SO_4, filtered and the solvent evaporated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1 to 1:1) afforded **5** (5.81 g, 76%) as white foam. $[\alpha]_D^{21}$ +3.4 (*c* = 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H, $C(CH_3)_3$), 1.74, 1.85 (m, 1H, H-2), 2.54 (d, $J_{4,OH}$ = 5.6 Hz, 1H, OH-4), 2.77 (d, $J_{3,OH}$ = 9.5 Hz, 1H, OH-3), 3.36 (m, 2H, H-1a, H-5), 3.85 (m, 2H, H-6), 3.98 (m, 2H, H-1b, H-4), 7.38-7.45, 7.66-7.71 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.17 (**C**$(CH_3)_3$), 26.81 (3C, C(**C**$H_3)_3$), 29.68 (C-2), 63.73 (C-6), 64.99 (C-1), 68.78 (C-4), 69.50 (C-3), 78.32 (C-5), 127.76, 127.77, 129.82, 132.93, 133.08, 135.54, 135.62 (12C, $2C_6H_5$); Anal. calcd for $C_{22}H_{30}O_4Si$: C 68.36; H 7.82; Found: C 68.00; H 7.83.

3,4-*O*-[(1*RS*)-4-(Allyl carboxylate)-benzylidene]-1,5-anhydro-2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-D-*lyxo*-hexitol (6a/b), (CRIV 4a/b), **(a:b 3:2).**

General procedure for methods A and B.

A soxhlet extractor containing activated powdered molecular sieves (3 Å) was conditioned at 95°C with dry CH_3CN (58 mL) for 20 h under argon. After cooling to r.t., dry **5** (1.2 eq) was added, followed by a solution of **1** or **2** (1 eq) in CH_3CN (2 mL). Toluene-4-sulfonicacid monohydrate (14.5 mg/mL in CH_3CN, 0.05 eq) was added to the mixture. The reaction was refluxed at 100°C under argon for 48 h. After cooling to r.t., the mixture was quenched by dropwise addition of Et_3N until pH 7 and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA/Et_3N 8:1, +1%) afforded a mixture of isomers **6a/b**.

Method A.

According to the general procedure **5** (170 mg, 43.8 μmol) was reacted with **1** (69 mg, 36 μmol) to afford **6a/b** (134 mg, 67%).

Method B.

According to the general procedure **5** (125 mg, 32.3 μmol) was reacted with **2** (64 mg, 27 μmol) to afford **6a/b** (141 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00 (s, 9H of **6a**, $C(CH_3)_3$), 1.07 (s, 9H of **6b**, $C(CH_3)_3$), 1.89 (m, 1H of **6b**, H-2a),1.90-1.95 (m, 2H of **6a**, H-2; 1H of **6b**, H-2b), 3.38-3.46 (m, 1H of **6a**, H-1a; 1H of **6b**, H-1a), 3.74 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 1H of **6a**, H-5), 3.82 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.3 Hz, 1H of **6b**, H-5), 3.83-4.02 (m, 3H of **6a**, H-1b, H6; 3H of **6b**, H-1b, H6), 4.24 (d, $J_{3,4}$ = 5.0 Hz, 1H of

6a, H-4), 4.29 (d, $J_{3,4}$ = 6.0 Hz, 1H of **6b**, H-4), 4.42, 4.54 (m, 1H of **6a**, H-3; 1H of **6b**, H-3), 4.83 (m, 2H of **6a**, $CH_2CHC\mathbf{H_2}O$; 2H of **6b**, $CH_2CHC\mathbf{H_2}O$), 5.30 (m, 1H of **6a**, Ha of $C\mathbf{H_2}CHCH_2O$; 1H of **6b**, Ha of $C\mathbf{H_2}CHCH_2O$), 5.41 (m, 1H of **6a**, Hb of $C\mathbf{H_2}CHCH_2O$; 1H of **6b**, Hb of $C\mathbf{H_2}CHCH_2O$), 5.91 (s, 1H of **6b**, CHO_2), 6.04 (m, 1H of **6a**, $CH_2C\mathbf{H}CH_2O$; 1H of **6b**, $CH_2C\mathbf{H}CH_2O$), 6.22 (s, 1H of **6a**, CHO_2), 7.28-7.42, 7.54-7.71, 8.06 (m, 14H of **6a**, $2C_6H_5$, C_6H_4; 14H of **6b**, $2C_6H_5$, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.21, 19.28 ($\mathbf{C}(CH_3)_3$, **6a/b**), 26.72, 26.82 (6C, $C(\mathbf{C}H_3)_3$, **6a/b**), 27.29, 29.16 (C-2, **6a/b**), 63.10, 63.21, 63.37, 64.07, 65.60 (C-1, **6a/b**; C-6, **6a/b**), 65.60 ($CH_2CH\mathbf{C}H_2O$, **6a/b**), 71.69 (C-3, **6b**), 72.03 (C-4, **6a**), 74.15 (C-4, **6b**), 75.33 (C-3, **6a**) 75.33 (C-5, **6b**), 76.28 (C-5, **6a**), 101.98 (CHO_2, **6a**), 102.92 (CHO_2, **6b**), 118.24 ($\mathbf{C}H_2CHCH_2O$, **6a/b**), 126.17, 126.63, 127.62, 127.67, 129.67, 129.69, 129.73, 130.51, 130.73, 132.19, 133.24, 133.41, 133.48, 135.52, 135.57, 135.59, 135.64, 142.94, 144.41 (38C, $CH_2\mathbf{C}HCH_2O$, **6a/b**; $2C_6H_5$, **6a/b**; C_6H_4, **6a/b**), 165.97, 165.98 (CO, **6a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{33}H_{38}NaO_6Si$ $[M+Na]^+$: 581.23; Found m/z: 581.22; IR (NaCl) ν 1723 vs (CO) (cm^{-1}); Anal. Calcd for $C_{33}H_{38}O_6Si$: C 70.94; H 6.85; Found: C 70.85; H 6.94.

1,5-Anhydro-2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-[(1*RS*)-4-(triethylammonium carboxylate)-benzylidene]-D-*lyxo*-hexitol (7a/b), (CRIV 5a/b), **(a:b 1:1).**

6a/b (1.41 g, 2.52 mmol) was dissolved in THF (21 mL) under argon. Acetylacetone (3.9 mL, 38 mmol) was added and the mixture was degassed by bubbling argon during 2 h. Triphenylphosphine (858 mg, 3.27 mmol) and $Pd(PPh_3)_4$ (581 mg, 0.503 mmol) were added with light exclusion. The reaction was stirred at r.t. for 21 h. After diluting with DCM (15 mL), the solution was filtered over a pad of celite and concentrated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 20:1, +1% Et_3N) afforded **7a/b** (1.38 g, 88%) as isomeric mixture. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00, 1.06 (2s, 9H of **7a**, $C(CH_3)_3$; 9H of **7b**, $C(CH_3)_3$), 1.29 (m, 9H of **7a**, $(C\mathbf{H}_3CH_2)_3N^+H$; 9H of **7b**, $(C\mathbf{H}_3CH_2)_3N^+H$), 1.80, 1.91 (m, 2H of **7a**, H-2; 2H of **7b**, H-2),3.09 (m, 6H of **7a**, $(CH_3C\mathbf{H}_2)_3N^+H$; 6H of **7b**, $(CH_3C\mathbf{H}_2)_3N^+H$), 3.37-3.46 (m, 1H of **7a**, H-1a; 1H of **7b**, H-1a), 3.72, 3.83 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.0 Hz, 1H of **7a**, H-5; 1H of **7b**, H-5), 3.85-4.02 (m, 3H of **7a**, H-1b, H6; 3H of **7b**, H-1b, H6), 4.27 (d, $J_{3,4}$ = 4.1 Hz, 1H of **7a**, H-4; 1H of **7b**, H-4), 4.39, 4.53 (m, 1H of **7a**, H-3; 1H

of **7b**, H-3), 5.90, 6.21 (s, 1H of **7a**, CHO_2; 1H of **7b**, CHO_2), 7.26-7.41, 7.47-7.52, 7.63-7.71, 8.08 (m, 14H of **7a**, $2C_6H_5$, C_6H_4; 14H of **7b**, $2C_6H_5$, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 8.73 (**C**$H_3CH_2NH^+$, **7a/b**), 19.17, 19.26 (**C**$(CH_3)_3$, **7a/b**), 26.81 (6C, C(**C**$H_3)_3$, **7a/b**), 29.45 (C-2, **7a/b**), 45.08 (CH_3**C**H_2NH^+, **7a/b**), 63.21, 63.27, 63.46, 64.07 (C-1, **7a/b**; C-6, **7a/b**), 71.44 (C-3, **7b**), 72.01 (C-4, **7a**), 72.63 (C-3, **7a**), 74.04 (C-4, **7b**), 76.46 (C-5, **7a/b**), 102.59 (CHO_2, **7a**), 103.59 (CHO_2, **7b**), 125.58, 126.07, 127.62, 127.65, 129.48, 129.54, 129.62, 133.16, 133.54, 135.52, 135.57, 135.61, 137.01, 141.47 (36C, $2C_6H_5$, **7a/b**; C_6H_4, **7a/b**), 172.28 (CO, **7a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{30}H_{34}NaO_6Si$ $[M+Na]^+$: 541.20; Found m/z: 541.22; IR (NaCl) ν 1600 s (CO) (cm^{-1}).

1,5-Anhydro-2-deoxy-3,4-*O*-[(1*RS*)-4-(triethylammonium carboxylate)-benzylidene]-D-*lyxo*-hexitol (8a/b), (CR IV 7), **(a:b 1:1).**

7a/b (245 mg, 0.395 mmol) was dissolved in THF (4 mL) and 1 M tetrabutylammonium fluoride solution in THF (0.79 mL, 0.79 mmol) was added. The mixture was stirred at r.t. for 22 h. The solvent was evaporated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 9:1, +1% Et_3N) gave **8a/b** (151 mg, quantitative) as yellowish foam. ^1H NMR (500 MHz, $CDCl_3$): δ 1.29 (m, 9H of **8a**, (C**H**$_3CH_2)_3NH^+$; 9H of **8b**, (C**H**$_3CH_2)_3NH^+$),1.88-1.92 (m, 2H of **8a**, H-2; 2H of **8b**, H-2), 3.05 (m, 6H of **8a**, (CH_3C**H**$_2)_3NH^+$; 6H of **8b**, (CH_3C**H**$_2)_3NH^+$), 3.37-3.50 (m, 1H of **8a**, H-1a; 1H of **8b**, H-1a), 3.64 (m, 1H of **8a**, H-5), 3.74-3.83 (m, 1H of **8a**, H6a; 2H of **8b**, H-5, H6a), 3.89-3.95 (m, 1H of **8a**, H-6b; 1H of **8b**, H-6b), 3.99-4.07 (m, 2H of **8a**, H-1b, H-4; 1H of **8b**, H-1b), 4.12 (m, 1H of **8b**, H-4), 4.37 (m, 1H of **8b**, H-3), 4.52 (m, 1H of **8a**, H-3), 5.86, 6.17 (s, 1H of **8a**, CHO_2; 1H of **8b**, CHO_2), 7.40, 7.49, 8.05 (m, 4H of **8a**, C_6H_4; 4H of **8b**, C_6H_4) ^{13}C NMR (125 MHz, $CDCl_3$): δ 8.72 (**C**$H_3CH_2)_3NH^+$, **8a/b**), 29.18, 29.69 (C-2, **8a/b**), 45.02 (CH_3**C**$H_2)_3NH^+$, **8a/b**), 63.10, 63.38, 63.46, 64.04 (C-1, **8a/b**; C-6, **8a/b**), 71.74 (C-3, **8b**), 72.86, 72.96 (C-3, **8a**; C-4, **8a**), 74.79 (C-4, **8b**), 75.40, 76.56 (C-5, **8a/b**), 102.72 (CHO_2, **8a**), 103.94 (CHO_2, **8b**), 125.63, 126.19, 129.56, 129.59, 137.28, 137.45, 139.60, 141.09 (12C, C_6H_5, **8a/b**), 171.98, 171.99 (CO, **8a/b**); Missing signals are due to overlapping or low resolution.

1,5-Anhydro-2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-isopropylidene-D-*lyxo*-hexitol (9), (CRV 2).

Compound **5** (1.00 g, 2.59 mmol) was dissolved in CH_3CN (53 mL) under argon. 2,2-dimethoxypropane (635 μL, 5.18 mmol) was added followed by camphor sulfonic acid in CH_3CN (1.0 mL, 21.5 mg/mL). After stirring the reaction vigorously at r.t. for 62 h, it was quenched by addition of Et_3N (30 mL). The solvents were evaporated and the resulting syrup was purified by silica gel chromatography (PE/EA 8:1, +1% Et_3N) to afford **9** (1.01 g, 92%) as white foam. $[\alpha]_D^{21}$ +4.1 (*c* = 0.70, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (s, 9H, $C(CH_3)_3$), 1.36,1.52 (2s, 6H, $C(CH_3)_2$), 1.75-1.87 (m, 2H, H-2), 3.36 (td, $J_{1a,1b}$ = 11.2 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 2.9 Hz, 1H, H-1a), 3.73 (m, 1H, H-5), 3.84 (dd, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 9.7 Hz, 1H, H-6a), 3.86-3.94 (m, 2H, H-1b, H-6b), 4.25 (m, 2H, H-3, H-4), 7.35-7.43, 7.67-7.72 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.41 (**C**$(CH_3)_3$), 26.38, 26.94, 28.37 (5C, C(**C**$H_3)_2$, C(**C**$H_3)_3$), 29.81 (C-2), 63.32 (C-6), 63.97 (C-1), 71.67, 71.85 (C-3, C-4), 75.96 (C-5), 108.98 (**C**$(CH_3)_2$), 127.72, 127.81, 129.75, 129.78, 133.64, 133.77, 135,72,135.81 (12C, $2C_6H_5$); Anal. calcd for $C_{25}H_{34}O_4Si$: C 70.38; H 8.03; Found: C 70.31; H 8.00.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-D-*lyxo*-hexitol (10), (CRV 3).

Compound **9** (1.01 g, 2.36 mmol) was dissolved in THF (50 mL) and 1 M tetrabutylammonium fluoride solution in THF (4.75 mL, 4.75 mmol) was added. The mixture was stirred at r.t. for 1 h. The solvent was evaporated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:3, +1% Et_3N) gave **10** (430 mg, 97%) as white foam. $[\alpha]_D^{21}$ -2.9 (*c* = 0.45, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.50 (2s, 6H, $C(CH_3)_2$), 1.78, 1.86 (m, 2H, H-2), 2.37 (t, $J_{6a,OH-6}$ = $J_{6b,OH-6}$ = 8.5 Hz, OH-6), 3.40 (ddd, $J_{1a,1b}$ = 11.5 Hz , $J_{1a,2a}$ = 10.9 Hz, $J_{1a,2b}$ = 2.9 Hz, 1H, H-1a), 3.68 (m, 1H, H-5), 3.75, 3.90 (m, 2H, H-6), 3.90 (m, 1H, H-6b), 3.99 (dt, $J_{1a,1b}$ = 11.6 Hz, $J_{1b,2a}$ = $J_{1b,2b}$ = 4.4 Hz, 1H, H-1b), 4.05 (dd, $J_{3,4}$ = 5.6 Hz, $J_{4,5}$ = 2.0 Hz, 1H, H-4), 4.26 (m, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.26 (**C**$(CH_3)_3$), 26.28, 28.09 (C(**C**$H_3)_2$), 29.30 (C-2), 63.53 (C-6), 63.71 (C-1), 71.81 (C-3), 72.95 (C-4), 75.62 (C-5), 109.40 (**C**$(CH_3)_2$); Anal. calcd for $C_9H_{16}O_4$: C 57.43; H 8.57; Found: C 57.10; H 8.67.

6-*S*-Acetyl-1,5-anhydro-2-deoxy-3,4-*O*-isopropylidene-6-thio-D-*lyxo*-hexitol (11), (CRV 4).

Compound **10** (372mg, 1.98 mmol) was dissolved under argon in THF (14.5 mL). To this solution triphenylphosphine (652 mg, 2.49 mmol) was added at -10°C followed by dropwise addition of DEAD (387 μL, 2.49 mmol). After stirring for 10 min, thiolacetic acid (185 μL, 2.49 mmol) was slowly added and stirring continued for 10 min at -10°C. Then the reaction was warmed to r.t.. After stirring for 39 h, the solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography (PE/EA 4:1, +0.5% Et_3N) to afford **11** (340 mg, 70%) as colorless oil. $[\alpha]_D^{21}$ +52.7 (*c* = 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34, 1.51 (2s, 6H, $C(CH_3)_2$), 1.75, 1.83 (m, 2H, H-2), 2.34 (s, 3H, SAc), 3.10 (dd, $J_{5,6a}$ = 8.8 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6a), 3.24 (dd, $J_{5,6b}$ = 4.7 Hz , $J_{6a,6b}$ = 13.9 Hz, 1H, H-6b), 3.37 (m, 1H, H-1a), 3.60 (m, 1H, H-5), 3.95 (dt, $J_{1a,1b}$ = 11.6 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 4.5 Hz, 1H, H-1b), 4.07 (dd, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 1.9 Hz, 1H, H-4), 4.23 (m, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.23, 28.04 ($C(\mathbf{C}H_3)_2$), 28.97 (C-2), 30.56 ($CO\mathbf{C}H_3$), 30.94 (C-6), 63.84 (C-1), 71.65 (C-3), 73.24 (C-4), 74.31 (C-5), 109.12 ($\mathbf{C}(CH_3)_2$), 195.89 (CO); IR (NaCl) ν 1694 vs (CO) (cm^{-1}); Anal. calcd for $C_{11}H_{18}O_4S$: C 53.64; H 7.37; Found: C 53.54; H 7.22.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*O*-methanesulfonyl-D-*lyxo*-hexitol (40), (CRVII 1).

Compound **10** (183 mg, 0.970 mmol) was dissolved in pyridine (87 mL). Methanesulfonyl chloride (0.15 mL, 1.9 mmol) was added dropwise at 0°C. After 15 min the reaction was warmed to r.t. and stirred for 1 h. The reaction was diluted with EtOAc (50 mL) and extracted with 1 N HCl (2 x 50 mL), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (PE/EA 2:1, +1% Et_3N) of the residue gave **40** (249 mg, 96%) as white foam. $[\alpha]_D^{21}$ +10.6 (*c* = 0.42, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.51 (2s, 6H, $C(CH_3)_2$), 1.80 (m, 1H, H-2a), 1.88 (m,1H, H-2b), 3.06 (s, 3H, SCH_3), 3.44 (td, $J_{1a,1b}$ = 11.2 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 3.0 Hz, 1H, H-1a), 3.92 (m, 1H, H-5), 3.99 (m, 1H, H-1b), 4.05 (d, $J_{3,4}$ = 5.6 Hz, 1H, H-4), 4.31 (m, 1H, H-3), 4.39 (m, 1H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.13, 27.82 ($C(\mathbf{C}H_3)_2$), 28.65 (C-2),

63.29 (C-1), 69.93 (C-6), 71.39 (C-3), 71.64 (C-4), 73.14 (C-5), 109.58 (**C**$(CH_3)_2$); MS (ESI): Calcd for $C_{10}H_{18}NaO_6S$ $[M+Na]^+$: 289.07; Found m/z: 288.99;

1,5-Anhydro-6-azido-2-deoxy-3,4-*O*-isopropylidene-D-*lyxo*-hexitol (41), (CRVII 2).

Compound **40** (365 mg, 1.37 mmol) was dissolved in dry DMF (55 mL) under argon. Sodium azide (446 mg, 6.86 mmol) was added followed by 15-crown-5 (0.11 mL, 0.55 mmol). The reaction was vigorously stirred under argon at 100°C for 3 days. The reaction mixture was then diluted at 0°C with EtOAc (30 mL) and washed with ice-cold water (2 x 30 mL) followed by extraction of the aqueous layer with EtOAc (4 x 40 mL). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and the resulting syrup was purified by silica gel chromatography (PE/EA 8:1, +1% Et_3N) to afford **41** (233 mg, 80%) as white solid. $[\alpha]_D^{21}$ +9.2 (*c* = 0.97, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34, 1.52 (2s, 6H, $C(CH_3)_2$), 1.78 (m, 1H, H-2a), 1.88 (m, 1H, H-2b), 3.37-3.45 (m, 2H, H-1a, H-6a), 3.53 (m, 1H, H-6b), 3.73 (m, 1H, H-5), 3.99 (m, 2H, H-1b, H-4), 4.28 (m, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.21, 28.01 (C(**C**$H_3)_2$), 29.00 (C-2), 52.04 (C-6), 63.64 (C-1), 71.54 (C-3), 72.37 (C-4), 74.49 (C-5), 109.39 (**C**$(CH_3)_2$); MS (ESI): Calcd for $C_9H_{15}N_3NaO_3$ $[M+Na]^+$: 236.10; Found m/z: 235.95; IR (NaCl) ν 2099 s (-N=N^+-N^-) (cm^{-1});

1,5-Anhydro-6-azido-2-deoxy-D-*lyxo*-hexitol (46), (CRVII 3).

Hexitol **41** (90 mg, 0.42 mmol) was stirred at 85°C in 80% aqueous AcOH (19 mL) for 3 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **46** (71 mg, 96%) as a white powder after a final liophilization from H_2O/dioxane (1:1). $[\alpha]_D^{21}$ +0.6 (*c* = 0.76, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.80-1.88 (m, 2H, H-2), 2.13 (m, 2H, OH-3, OH-4), 3.37 (dd, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H-6a), 3.44 (m, 2H, H-1a, H-5), 3.60 (dd, $J_{5,6b}$ = 7.5 Hz, $J_{6a,6b}$ = 12.5 Hz, 1H, H-6b), 3.76 (m, 2H, H-3, H-4), 4.05 (dd, $J_{1a,1b}$ = 11.7 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 4.3 Hz, 1H, H-1b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.64 (C-2), 52.42 (C-6), 63.32 (C-1), 69.54 (2C, C-3, C-4), 77.71 (C-5); MS (ESI): Calcd for $C_6H_{11}N_3NaO_3$ $[M+Na]^+$: 196.07; Found m/z: 195.87; IR (NaCl) ν 2099 s (-N=N^+-N^-) (cm^{-1});

3,4-*O*-[(1*RS*)-4-(Allyl carboxylate)-benzylidene]-1,5-anhydro-6-azido-2-deoxy-D-*lyxo*-hexitol (47a/b), (CRVII 4a/b), **(a:b 1:1).**

A soxhlet extractor with activated powdered molecular sieves (3 Å, was conditioned at 95°C with dry CH_3CN (65 mL) for 20 h under argon. After cooling to r.t., dry **46** (164 mg, 0.947 mmol) and **2** (193 mg, 0.816 mmol), both dissolved in CH_3CN (5 mL), were added. Toluene-4-sulfonicacid monohydrate (0.73 mL of 11 mg/mL solution in CH_3CN) was added to the mixture. The mixture was refluxed at 105 °C. After 20 h no more **2** was detected by TLC but instead **1** (the aldehyde form). Additional 0.7 eq of **2** (135 mg, 0.571 mmol) were added and the reaction was stirred for another 16 h. After cooling to r.t., the reaction was quenched by addition of Et_3N until pH 7 and the mixture was concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 8:1 to 4:1, +1% Et_3N) afforded **47a/b** (124 mg, 44%). It was possible to separate the two isomers from the 1:1 mixture. **47a**: $[\alpha]_D^{21}$ +51.4 (*c* = 0.53, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.87-2.04 (m, 2H, H-2), 3.39-3.48 (m, 2H, H-1a, H-6a), 3.66-3.74 (m, 2H, H-5, H-6b), 3.99 (m, 1H, H-4), 4.09 (m, 1H, H-1b), 4.57 (m, 1H, H-3), 4.83 (m, 2H, $CH_2CHC\mathbf{H_2}O$), 5.29 (m, 1H, Ha of $C\mathbf{H_2}CHCH_2O$), 5.41 (m, 1H, Hb of $C\mathbf{H_2}CHCH_2O$), 6.04 (m, 1H, $CH_2C\mathbf{H}CH_2O$), 6.21 (s, 1H, CHO_2), 7.51, 8.07 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.74 (C-2), 52.23 (C-6), 63.97 (C-1), 65.66 ($CH_2CH\mathbf{C}H_2O$), 72.63, 72.88 (C-3, C-4), 75.26 (C-5), 102.21 (CHO_2), 118.33 ($\mathbf{C}H_2CHCH_2O$), 126.17, 129.83, 130.79, 143.77 (6C, C_6H_4), 132.16 ($CH_2\mathbf{C}HCH_2O$); IR (NaCl) ν 2104 s (-N=N$^+$-N$^-$), 1720 vs (CO) (cm^{-1}). **47b**: $[\alpha]_D^{21}$ -40.6 (*c* = 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.81 (m, 1H, H-2a), 1.95 (m, 1H, H-2b), 3.45 (dd, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.8 Hz, 1H, H-6a), 3.54 (td, $J_{1a,1b}$ = 11.8 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 3.7 Hz, H-1a), 3.66 (dd, $J_{5,6b}$ = 8.0 Hz, $J_{6a,6b}$ = 12.7 Hz, 1H, H-6b), 3.81 (m, 1H, H-5), 4.03 (m, 1H, H-1b), 4.12 (m, 1H, H-4), 4.46 (m, 1H, H-3), 4.83 (m, 2H, $CH_2CHC\mathbf{H_2}O$), 5.29 (m, 1H, Ha of $C\mathbf{H_2}CHCH_2O$), 5.41 (m, 1H, Hb of $C\mathbf{H_2}CHCH_2O$), 5.92 (s, 1H, CHO_2), 6.04 (m, 1H, $CH_2C\mathbf{H}CH_2O$), 7.61, 8.09 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.42 (C-2), 51.90 (C-6), 63.22 (C-1), 65.65 ($CH_2CH\mathbf{C}H_2O$), 71.81 (C-3), 73.96 (C-5), 74.49 (C-4), 103.29 (CHO_2), 118.28 ($\mathbf{C}H_2CHCH_2O$), 126.72, 129.80, 131.02, 142.41 (6C, C_6H_4), 132.16 ($CH_2\mathbf{C}HCH_2O$), 165.90 (CO); IR (NaCl) ν 2103 s (-N=N$^+$-N$^-$), 1721 vs (CO) (cm^{-1}).

1,5-Anhydro-6-azido-2-deoxy-3,4-*O*-[(1*RS*)-4-(triethylammonium carboxylate)-benzylidene]-D-*lyxo*-hexitol (49a/b), (CRVII 7), **(a:b 1:1).**

To a solution of **47a/b** (114 mg, 0.330 mmol) in H_2O/dioxane (1:1, 11 mL) was added lithium hydroxide (80.6 mg, 3.30 mmol). The reaction was stirred at r.t. for 17 h. The solvents were evaporated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 20:1 to 9:1, +1% Et_3N) afforded the two isomers **49a/b** (103 mg, 77%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (m, 9H of **49a**, ($CH_3CH_2)_3NH^+$; 9H of **49b**, ($CH_3CH_2)_3NH^+$), 1.95 (m, 2H of **49a**, H-2; 2H of **49b**, H-2), 3.10 (m, 6H of **49a**, ($CH_3CH_2)_3NH^+$; 6H of **49b**, ($CH_3CH_2)_3NH^+$), 3.37-3.51 (m, 2H of **49a**, H-1a, H-6a; 2H of **49b**, H-1a, H-6a), 3.52-3.70 (m, 1H of **49a**, H-6b; 2H of **49b**, H-5, H-6b), 3.80 (m, 1H of **49a**, H-5), 3.98-4.09 (m, 2H of **49a**, H-1b, H-4; 2H of **49b**, H-1b, H-4), 4.42 (m, 1H of **49b**, H-3), 4.53 (m, 1H of **49a**, H-3), 5.90 (s, 1H of **49b**, CHO_2), 6.19 (s, 1H of **49a**, CHO_2), 7.43, 7.53, 8.07 (m, 4H of **49a**, C_6H_4; 4H of **49b**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 8.64 (($CH_3CH_2)_3NH^+$, **49a/b**),28.61, 29.70 (C-2, **49a/b**), 45.18 (($CH_3CH_2)_3NH^+$, **49a/b**), 52.37, 52.94 (C-6, **49a/b**), 63.27, 63.98 (C-1, **49a/b**), 71.61 (C-3, **49b**), 72.55, 72.73 (C-3, **49a**; C-4, **49a**), 74.01 (C-5, **49b**), 74.41 (C-4, **49b**), 75.38 (C-5, **49a**), 102.73, 103.88 (CHO_2, **49a/b**), 125.65, 126.26, 129.68, 136.46, 136.68, 139.80, 141.28 (12C, C_6H_4, **49a/b**); MS (ESI): Calcd for $C_{14}H_{15}N_3NaO_5$ $[M+Na]^+$: 328.09; Found m/z: 328.09; IR (NaCl) ν 2104 s ($-N=N^+-N^-$), 1705 vs (CO) (cm^{-1}).

***Galacto*-mimics modified at the 6-position.**

General procedure for *S*-deacetylations and Michael addition or nucleophilic substitution (from **11**).

Compound **11** (1.0 eq, 60 mg scale) was dissolved in freshly degassed DMF (1.9 mL) under argon. The solution was again degassed by bubbling argon through it for 40 min. Hydrazine acetate (1.16 eq) was added quickly under flowing argon. After stirring the mixture for 4 h at r.t., freshly degassed Et_3N (1.30 eq) was added. After 10-15 min, the corresponding α,β-unsaturated ketone or α-halo ketone (1.50 eq) was added to the mixture. For solid reagents a solution in DMF (200 mg/mL) was prepared and degassed prior to its addition. After 24 h to 6 d (depending on the reactants), the mixture was diluted with EtOAc (5 mL) and extracted with 1 N HCl (3 mL) and saturated aqueous $NaHCO_3$ (2 x 3 mL). The organic layer was dried with

Na_2SO_4, filtered and concentrated. Purifications were done by silica gel chromatography (PE/EA 4:1 to 2:1, +0.5% Et_3N).

Bis [1,5-anhydro-3,4-*O*-isopropylidene-D-*lyxo*-hexitol-6-yl]-disulphide (15), (CRV 6).

Obtained as secondary product due to oxidation during Michael additions and nucleophilic substitutions from 11. Separated during purification processes of such reactions. $[\alpha]_D^{21}$ -97.1 (*c* = 0.54, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33, 1.50 (2s, 6H, $C(CH_3)_2$), 1.67-1.88 (m, 2H, H-2), 2.95-3.05 (m, 2H, H-6), 3.41 (m, 1H, H-1a), 3.84 (m, 1H, H-5), 3.97 (m, 1H, H-1b), 4.12 (d, $J_{3,4}$ = 5.6 Hz, 1H, H-4), 4.27 (m, 1H, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.23, 28.06 (4C, $C(CH_3)_2$), 29.04 (C-2), 40.02 (C-6), 63.85 (C-1), 71.68 (C-3), 72.91 (C-4), 73.79 (C-5), 109.04, 109.60 ($C(CH_3)_2$); MS (ESI): Calcd for $C_{18}H_{30}NaO_6S_2$ $[M+Na]^+$: 445.11; Found m/z: 445.00; Anal. calcd for $C_{18}H_{30}O_6S_2$: C 53.18; H 7.44; Found : C 53.49; H 7.36.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-3-oxo-cyclohexyl]-6-thio-D-*lyxo*-hexitol (16a/b), (CRV5a/b), **(a:b 1:1).**

According to the general procedure, **11** (60.5 mg, 0.246 mmol) was deprotected with hydrazine acetate (26.4 mg, 0.287 mmol) and reacted with 2-cyclohexen-1-one (36 μL, 0.38 mmol) for 75 h. After work-up and chromatographic purification **16a/b** (38 mg, 51%) was obtained as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34, 1.52 (2s, 6H of **16a**, $C(CH_3)_2$; 6H of **16b**, $C(CH_3)_2$), 1.71-1.87, 2.12-2.18, 2.29-2.42, 2.73-2.82 (m, 11H of **16a**, H-2, H-6a, 8H of C_6H_9; 11H of **16b**, H-2, H-6a, 8H of C_6H_9), 2.91 (m, 1H of **16a**, H-6b; 1H of **16b**, H-6b), 3.16 (m, 1H of **16a**, H-1'; 1H of **16b**, H-1'), 3.39 (m, 1H of **16a**, H-1a; 1H of **16b**, H-1a), 3.64 (m, 1H of **16a**, H-5; 1H of **16b**, H-5), 3.97 (m, 1H of **16a**, H-1b; 1H of **16b**, H-1b), 4.12 (m, 1H of **16a**, H-4; 1H of **16b**, H-4), 4.26 (m, 1H of **16a**, H-3; 1H of **16b**, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.20, 24.22 (CH_2, **16a/b**), 26.23, 26.26 ($C(\mathbf{C}H_3)_2$, **16a/b**), 28.09 ($C(\mathbf{C}H_3)_2$, **16a/b**), 29.05, 29.07 (C-2, **16a/b**), 31.76, 31.86, 31.92, 31.99 (C-6, **16a/b**; CH_2, **16a/b**), 41.04, 41.05 (CH_2, **16a/b**), 43.59, 43.68 (C-1', **16a/b**), 48.28, 48.47 (CH_2, **16a/b**), 63.97, 64.01 (C-1, **16a/b**), 71.79 (C-3, **16a/b**), 72.93, 72.99 (C-4, **16a/b**), 75.81, 75.82 (C-5, **16a/b**), 109.15, 109.13 ($\mathbf{C}(CH_3)_2$, **16a/b**), 208.92, 208.99 (CO, **16a/b**); Missing signals are due to overlapping or low resolution. IR (NaCl) ν 1715 vs (CO)

(cm^{-1}); Anal. calcd for $C_{15}H_{24}O_4S$: C 59.97; H 8.05; O 21.30; Found: C 59.94; H 8.10; O 21.44.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-3-oxo-cyclopentyl]-6-thio-D-*lyxo*-hexitol (17a/b), (CRV 9a/b), **(a:b 1:1).**

According to the general procedure, **11** (299 mg, 1.22 mmol) was deprotected with hydrazine acetate (130 mg, 1.41 mmol) and reacted with 2-cyclopenten-1-one (157 μL, 1.83 mmol) for 5 d. After work-up and purification **17a/b** (52 mg, 14%) was obtained as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35, 1.53 (2s, 6H of **17a**, $C(CH_3)_2$; 6H of **17b**, $C(CH_3)_2$), 1.74-1.88 (m, 2H of **17a**, H-2; 2H of **17b**, H-2), 1.97, 2.18-2.27, 2.38-2.50, 2.64 (m, 6H of **17a**, 6H of C_5H_7; 6H of **17b**, 6H of C_5H_7), 2.82, 2.93 (m, 2H of **17a**, H-6; 2H of **17b**, H-6), 3.40 (m, 1H of **17a**, H-1a; 1H of **17b**, H-1a), 3.58 (m, 1H of **17a**, H-1'; 1H of **17b**, H-1'), 3.67 (m, 1H of **17a**, H-5; 1H of **17b**, H-5), 3.98 (m, 1H of **17a**, H1b; 1H of **17b**, H1b), 4.12 (d, $J_{3,4}$ = 3.9 Hz, 1H of **17a**, H-4; 1H of **17b**, H-4), 4.27 (m, 1H of **17a**, H-3; 1H of **17b**, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.20, 24.22 (CH_2, **17a/b**), 26.25, 28.08 ($C(\mathbf{C}H_3)_2$, **17a/b**), 29.02 (C-2, **17a/b**), 29.98, 30.07 (CH_2, **17a/b**), 32.64, 32.70 (CH_2, **17a/b**), 37.19 (C-6, **17a/b**), 41.24 (C-1', **17a/b**), 45.81, 45.95 (CH_2, **17a/b**), 63.90 (C-1, **17a/b**), 71.69 (C-3, **17a/b**), 72.94, 72.97 (C-4, **17a/b**), 75.66, 75.74 (C-5, **17a/b**), 109.08, ($\mathbf{C}(CH_3)_2$, **17a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{14}H_{22}NaO_4S$ $[M+Na]^+$: 309.11; Found m/z: 309.16; IR (NaCl) ν 1744 vs (CO) (cm^{-1}).

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-2-oxo-cyclohexyl]-6-thio-D-*lyxo*-hexitol (18a/b), (CRV 14a/b), **(a:b 1:1).**

According to the general procedure, **11** (155 mg, 0.629 mmol) was deprotected with hydrazine acetate (68 mg, 0.74 mmol) and reacted with 2-chlorocyclohexanone (110 μL, 0.944 mmol) for 32 h. After work-up and purification as in the general procedure, an additional purification by silica gel chromatography was applied [a) Hexane, b) PE/EA 2:1, +0.5% Et_3N], **18a/b** (148 mg, 78%) was obtained as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31, 1.49 (s, 6H of **18a**, $C(CH_3)_2$; 6H of **18b**, $C(CH_3)_2$), 1.65, 1.70-1.90, 1.95, 2.03, 2.15-2.26 (m, 10H of **18a**, H-2, 8H of C_6H_9; 10H of **18b**, H-2, 8H of C_6H_9), 2.70 (m, 1H of **18a**, H-6a; 1H of **18b**, H-6a), 2.82-2.99 (m, 1H of **18a**, H-6b; 1H of **18b**, H-6b), 3.33-3.46 (m, 2H of **18a**, H-1a, H-1'; 2H of **18b**, H-1a, H-1'), 3.61 (m, 1H of **18a**, H-5), 3.66 (m, 1H of **18b**, H-5), 3.94 (m, 1H of **18a**, H1b;

1H of **18b**, H1b), 4.04 (m, 1H of **18a**, H-4; 1H of **18b**, H-4), 4.22 (m, 1H of **18a**, H-3; 1H of **18b**, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.86, 21.90, 26.27, 26.96, 26.99, 28.09, 29.07, 29.11, 33.08, 33.25, 33.28, 34.12, 37.88 (18C, C-2, **18a/b**; C-6, **18a/b**; 5CH_2 of C_6H_9, **18a/b**; C(**C**H_3)$_2$, **18a/b**), 52.35, 52.84 (C-1', **18a/b**), 63.87, 63.92 (C-1, **18a/b**), 71.67, 71.69 (C-3, **18a/b**), 73.04, 73.19 (C-4, **18a/b**), 74.23 (C-5, **18b**), 74.91 (C-5, **18a**), 109.02 (**C**$(CH_3)_2$, **18a/b**), 208.39, 208.42 (CO, **18a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{15}H_{24}NaO_4S$ $[M+Na]^+$: 323.13; Found m/z: 323.10; IR (NaCl) ν 1704 vs (CO) (cm^{-1}).

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-2-oxo-cyclopentyl]-6-thio-D-*lyxo*-hexitol (19a/b), (CRV 10a/b), **(a:b 1:1).**

According to the general procedure, **11** (90.6 mg, 0.368 mmol) was deprotected with hydrazine acetate (39.6 mg, 0.430 mmol) and reacted with 2-chloropentan-1-one (57 μL, 0.56 mmol) for 25 h. After work-up and purification **19a/b** (80 mg, 76%) was obtained as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.50 (s, 6H of **19a**, C$(CH_3)_2$; 6H of **19b**, C$(CH_3)_2$), 1.62-1.91, 2.04-2.29, 2.45, 2.83-2.94 (m, 8H of **19a**, H-2, 6H of C_5H_7; 8H of **19b**, H-2, 6H of C_5H_7), 3.09 (m, 1H of **19a**, H-6a; 2H of **19b**, H-6a, H-6b), 3.27 (m, 1H of **19a**, H-6b), 3.40 (m, 1H of **19a**, H-1'; 1H of **19b**, H-1'), 3.67 (m, 1H of **19a**, H-1a; 1H of **19b**, H-1a), 3.74 (m, 1H of **19a**, H-5), 3.94 (m, 1H of **19b**, H-5), 4.03 (m, 1H of **19a**, H-1b; 1H of **19b**, H-1b), 4.23 (m, 1H of **19a**, H-4, 1H of **19b**, H-4), 4.26 (m, 1H of **19a**, H-3; 1H of **19b**, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.38, 20.45 (CH_2, **19a/b**), 26.26, 28.10 (C(**C**H_3)$_2$, **19a/b**), 29.07, 29.12 (C-2, **19a/b**), 30.27, 30.34, 32.48, 32.52 (CH_2, **19a/b**), 36.00, 36.03 (C-6, **19a/b**), 47.40, 48.17 (C-1', **19a/b**), 63.84, 63.95 (C-1, **19a/b**), 71.69 (C-3, **19a/b**), 73.14, 73.20 (C-4, **19a/b**), 74.02, 75.40 (C-5, **19a/b**), 109.03 (**C**$(CH_3)_2$, **19a/b**), 214.03, 214.34 (CO, **19a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{14}H_{22}NaO_4S$ $[M+Na]^+$: 309.11; Found m/z: 309.10; IR (NaCl) ν 1731 vs (CO) (cm^{-1}); Anal. calcd for $C_{15}H_{24}O_4S$: C 58.72; H 7.74; Found: C 58.83, H 7.70.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-(2-oxo-2-phenyl-ethyl)-6-thio-D-*lyxo*-hexitol (20), (CRV 17).

According to the general procedure, **11** (121 mg, 0.492 mmol) was deprotected with hydrazine acetate (55 mg, 0.59 mmol) and reacted with 2-chloroacetophenone (114 mg, 0.738 mmol) for 24 h. After work-up and purification **20** (122 mg, 77%) was

obtained. $[\alpha]_D^{21}$ +10.7 (*c* = 0.28, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.31, 1.49 (2s, 6H, $C(CH_3)_2$), 1.67-1.84 (m, 2H, H-2), 2.81 (dd, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 14.0 Hz, 1H, H-6a), 2.95 (dd, $J_{5,6b}$ = 8.2 Hz, $J_{6a,6b}$ = 14.0 Hz, 1H, H-6b), 3.35 (td, $J_{1a,1b}$ = 11.2 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 2.7 Hz, 1H, H-1a), 3.71 (m, 1H, H-5), 3.92 (m, 3H, H-1b, H-1'), 4.06 (d, $J_{3,4}$ = 4.3 Hz, 1H, H-4), 4.24 (m, 1H, H-3), 7.47, 7.57, 7.97 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.23, 28.08 ($C(\mathbf{C}H_3)_2$), 29.04 (C-2), 33.80 (C-6), 38.16 (C-1'), 63.82 (C-1), 71.67 (C-3), 73.13 (C-4), 74.86 (C-5), 109.04 ($\mathbf{C}(CH_3)_2$), 128.68, 128.73, 133.40, 135.35 (6C, C_6H_5), 194.72 (CO); MS (ESI): Calcd for $C_{17}H_{22}NaO_4S$ $[M+Na]^+$: 345.11; Found m/z: 345.08; IR (NaCl) ν 1674 vs (CO) (cm^{-1}); Anal. calcd for $C_{17}H_{22}O_4S$ + 0.25 H_2O: C 62.46, H 6.94; Found: C 62.49, H 6.66.

1,5-Anhydro-2-deoxy-6-*S*-[2-(4-fluorophenyl)-2-oxo-ethyl]-3,4-*O*-isopropylidene-6-thio-D-*lyxo*-hexitol (21), (CRV 18).

According to the general procedure, **11** (113 mg, 0.460 mmol) was deprotected with hydrazine acetate (50 mg, 0.54 mmol) and reacted with 2-chloro-4'-fluoroacetophenone (120 mg, 0.689 mmol) for 24 h. After work-up and purification **21** (105 mg, 67%) was obtained as white solid. $[\alpha]_D^{21}$ +8.1 (*c* = 1.08, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.31, 1.50 (2s, 6H, $C(CH_3)_2$), 1.70-1.86 (m, 2H, H-2), 2.80 (dd, $J_{5,6a}$ = 5.2 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6a), 2.95 (dd, $J_{5,6b}$ = 8.3 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6b), 3.36 (td, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 2.8 Hz, 1H, H-1a), 3.71 (m, 1H, H-5), 3.89 (m, 3H, H-1b, H-1'), 4.06 (m, 1H, H-4), 4.24 (m, 1H, H-3), 7.14, 8.01 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.24, 28.07 ($C(\mathbf{C}H_3)_2$), 29.02 (C-2), 33.82 (C-6), 38.05 (C-1'), 63.82 (C-1), 71.67 (C-3), 73.16 (C-4), 74.87 (C-5), 109.08 ($\mathbf{C}(CH_3)_2$), 115.74, 115.92, 131.41, 131.49, 131.72 (6C, C_6H_4), 193.20 (CO); MS (ESI): Calcd for $C_{17}H_{21}FNaO_4S$ $[M+Na]^+$: 363.10; Found m/z: 363.06; IR (NaCl) ν 1674 vs (CO) (cm^{-1}); Anal. calcd for $C_{17}H_{21}FO_4S$: C 59.98; H 6.22; Found: C 60.19; H 6.39.

1,5-Anhydro-6-*S*-[2-(4-chlorophenyl)-2-oxo-ethyl]-2-deoxy-3,4-*O*-isopropylidene-6-thio-D-*lyxo*-hexitol (22), (CRV 19).

According to the general procedure, **11** (77 mg, 0.31 mmol) was deprotected with hydrazine acetate (34 mg, 0.37 mmol) and reacted with 2-bromo-4'-chloroacetophenone (112 mg, 0.470 mmol) for 24 h. After work-up and purification **22** (105 mg, 94%) was obtained as colorless oil. $[\alpha]_D^{21}$ +3.1 (*c* = 0.40, $CHCl_3$); ^1H NMR

(500 MHz, $CDCl_3$): δ 1.31, 1.49 (2s, 6H, $C(CH_3)_2$), 1.72-1.81 (m, 2H, H-2), 2.78 (m, 1H, H-6a), 2.94 (m, 1H, H-6b), 3.35 (m, 1H, H-1a), 3.70 (m, 1H, H-5), 3.89 (m, 3H, H-1b, H-1'), 4.05 (m, 1H, H-4), 4.18 (m, 1H, H-3), 7.44, 7.91 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.23, 28.06 ($C(\mathbf{C}H_3)_2$), 29.00 (C-2), 33.80 (C-6), 38.04 (C-1'), 63.80 (C-1), 71.66 (C-3), 73.15 (C-4), 74.88 (C-5), 109.07 ($\mathbf{C}(CH_3)_2$), 129.01, 130.18, 133.63, 139.86 (6C, C_6H_4), 193.50 (CO); MS (ESI): Calcd for $C_{17}H_{21}ClNaO_4S$ $[M+Na]^+$: 379.07; Found m/z: 379.01; IR (NaCl) ν 1674 vs (CO) (cm^{-1}); Anal. calcd for $C_{17}H_{21}ClO_4S$: C 57.22; H 5.93; Found: C 57.34; H 5.95.

1,5-Anhydro-2-deoxy-6-*S*-[2-(2,5-dimethoxy-phenyl)-2-oxo-ethyl]-3,4-*O*-isopropylidene-6-thio-D-*lyxo*-hexitol (23), (CRV 23).

According to the general procedure, **11** (66 mg, 0.27 mmol) was deprotected with hydrazine acetate (29 mg, 0.32 mmol) and reacted with 2-bromo-2',5'-dimethoxy-acetophenone (108 mg, 0.404 mmol) for 22 h. After work-up and purification **23** (78 mg, 75%) was obtained as colorless oil. $[\alpha]_D^{21}$ +10.6 (*c* = 0.40, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.31, 1.49 (2s, 6H, $C(CH_3)_2$), 1.74 (m, 1H, H-2a), 1.83 (m, 1H, H-2b), 2.78 (dd, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 13.8 Hz, 1H, H-6a), 2.86 (dd, $J_{5,6b}$ = 7.9 Hz, $J_{6a,6b}$ = 13.8 Hz, 1H, H-6b), 3.36 (m, 1H, H-1a), 3.68 (m, 1H, H-5), 3.80, 3.87 (2s, 6H, $2OCH_3$), 3.95 (m, 3H, H-1b, H-1'), 4.07 (m, 1H, H-4), 4.22 (m, 1H, H-3), 6.91, 7.05, 7.36 (m, 3H, C_6H_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.09, 26.21 ($C(\mathbf{C}H_3)_2$), 29.13 (C-2), 33.47 (C-6), 43.02 (C-1'), 55.84 (OCH_3), 56.13 (OCH_3), 63.88 (C-1), 71.69 (C-3), 73.02 (C-4), 74.61 (C-5), 108.97($\mathbf{C}(CH_3)_2$), 113.17, 114.56, 120.91, 126.16, 153.28, 153.58 (C_6H_3), 195.69 (CO); MS (ESI): Calcd for $C_{19}H_{26}NaO_6S$ $[M+Na]^+$: 405.13; Found m/z: 405.03; IR (NaCl) ν 1674 vs (CO) (cm^{-1}); Anal. calcd for $C_{19}H_{26}O_6S$: C 59.67; H 6.85; Found : C 59.69; H 6.85.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-(2-napht-2'-yl-2-oxo-ethyl)-6-thio-D-*lyxo*-hexitol (24), (CRV 24).

According to the general procedure, **11** (64 mg, 0.26 mmol) was deprotected with hydrazine acetate (29 mg, 0.31 mmol) and reacted with 2-bromo-2'-acetonaphtone (99 mg, 0.39 mmol) for 25 h. After work-up and purification according to the general procedure an additional purification by silica gel chromatography (PE/EA 4:1, +0.5% Et_3N) was done and **24** (59 mg, 60%) was obtained as colorless oil. $[\alpha]_D^{21}$ +6.5 (*c* =

0.58, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.31, 1.44 (2s, 6H, $C(CH_3)_2$), 1.71-1.84 (m, 2H, H-2), 2.85 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 13.6 Hz, 1H, H-6a), 3.00 (dd, $J_{5,6b}$ = 8.2 Hz, $J_{6a,6b}$ = 13.7 Hz, 1H, H-6b), 3.36 (m, 1H, H-1a), 3.75 (m, 1H, H-5), 3.92 (m, 1H, H-1b), 4.06 (m, 3H, H-4, H-1'), 4.24 (m, 1H, H-3), 7.59, 7.81-8.04, 8.50 (m, 7H, $C_{10}H_7$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.24, 28.09 ($C(\mathbf{C}H_3)_2$), 29.06 (C-2), 33.88 (C-6), 38.27 (C-1'), 63.85 (C-1), 71.68 (C-3), 73.17 (C-4), 74.96 (C-5), 109.06 ($\mathbf{C}(CH_3)_2$), 124.27, 126.88, 127.81, 128.58, 128.70, 129.66, 130.57, 132.49, 132.68, 135.71 ($C_{10}H_7$), 194.75 (CO); MS (ESI): Calcd for $C_{21}H_{24}NaO_4S$ $[M+Na]^+$: 395.13; Found m/z: 395.03; IR (NaCl) ν 1674 vs (CO) (cm^{-1}). Anal. calcd for $C_{21}H_{24}O_4S$: C 66.12; H 6.61; Found : C 66.28; H 6.54.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(2*RS*)-1-oxo-indan-2yl]-6-thio-D-*lyxo*-hexitol (25a/b), (CRV 26a/b) **(a:b 1:1).**

According to the general procedure, **11** (107 mg, 0.434 mmol) was deprotected with hydrazine acetate (48 mg, 0.52 mmol) and reacted with 2-bromo-1-indanone (153 mg, 0.651 mmol) for 26 h. After work-up and purification according to the general procedure an additional purification by silica gel chromatography (PE/EA 4:1, +0.5% Et_3N) was done and **25a/b** (95 mg, 65%) was obtained as colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.33, 1.50 (4s, 6H of **25a**, $C(CH_3)_2$; 6H of **25b**, $C(CH_3)_2$), 1.72-1.88 (m, 2H of **25a**, H-2; 2H of **25b**, H-2), 2.96-3.02 (m, 2H of **25a**, H-6a, H-3'a; 2H of **25b**, H-6a, H-3'a), 3.14 (dd, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H of **25a**, H-6b), 3.22 (dd, $J_{5,6b}$ = 8.2 Hz, $J_{6a,6b}$ = 13.7 Hz, 1H of **25b**, H-6b), 3.34 (m, 1H of **25b**, H-1a), 3.43 (m, 1H of **25a** , H-1a), 3.60 (m, 1H of **25a**, H-3'b; 1H of **25b**, H-3'b), 3.71-3.78 (m, 1H of **25a**, H-2'; 2H of **25b**, H-5, H-2'), 3.86 (m, 1H of **25a**, H-5; 1H of **25b**, H-1b), 3.98 (m, 1H of **25a**, H-1b), 4.14 (m, 1H of **25a**, H-4; 1H of **25b**, H-4), 4.26 (m, 1H of **25a**, H-3; 1H of **25b**, H-3), 7.39, 7.52, 7.77 (m, 4H of **25a**, C_6H_4; 4H of **25b**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.25, 28.08 (4C, $C(\mathbf{C}H_3)_2$, **25a/b**), 29.02, 29.11 (C-2, **25a/b**), 32.45, 32.52 (C-6, **25a/b**), 34.48, 34.60 (C-3', **25a/b**), 45.61, 46.44 (C-2', **25a/b**), 63.72, 63.92 (C-1, **25a/b**), 71.69 (C-3, **25a/b**), 73.18 (C-4, **25a/b**), 74.33 (C-5, **25a**), 75.56 (C-5, **25b**), 109.03 ($\mathbf{C}(CH_3)_2$, **25a/b**), 124.59, 124.65, 126.35, 126.39, 127.81, 127.87, 135.14, 135.19, 135.25, 152.04 (12C, C_6H_4, **25a/b**), 203.13, 203.38 (CO, **25a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{18}H_{22}NaO_4S$ $[M+Na]^+$: 357.11; Found m/z: 357.06; IR (NaCl) ν 1713

vs (CO) (cm^{-1}); Anal. calcd for $C_{18}H_{22}O_4S$ + 0.25 H_2O: C 63.79; H 6.69; Found : C 64.11; H 6.81.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-3-(4-methoxy-phenyl)-3-oxo-1-phenyl-propyl]-6-thio-D-*lyxo*-hexitol (26),(CRV 11a/b), **(a:b 1:1).**

According to the general procedure, **11** (271 mg, 1.10 mmol) was deprotected with hydrazine acetate (119 mg, 1.28 mmol) and reacted with 4'-methoxychalcone (408 mg, 1.66 mmol) for 6 d. After work-up and purification **26a/b** (98 mg, 28%) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30, 1.38, 1.47, 1.51 (4s, 6H of **26a**, $C(CH_3)_2$; 6H of **26b**, $C(CH_3)_2$), 1.71-1.80 (m, 2H of **26a**, H-2; 2H of **26b**, H-2**)**, 2.61-2.76 (m, 2H of **26a**, H-6; 2H of **26b**, H-6), 3.30 (m, 1H of **26a**, H-1a; 1H of **26b**, H-1a), 3.44-3.54 (m, 3H of **26a**, H-5, H-2'; 3H of **26b**, H-5, H-2'), 3.86 (s, 3H of **26a**, OCH_3; 3H of **26b**, OCH_3), 3.92 (m, 1H of **26a**, H-1b; 1H of **26b**, H-1b), 4.04 (d, $J_{3,4}$ = 4.6 Hz, 1H of **26a**, H-4), 4.15 (d, $J_{3,4}$ = 3.8 Hz, 1H of **26b**, H-4), 4.20 (m, 1H of **26a**, H-3; 1H of **26b**, H-3), 4.63 (m, 1H of **26a**, H-1'; 1H of **26b**, H-1'), 6.90, 7.21, 7.30, 7.43, 7.89 (m, 9H of **26a**, C_6H_5, C_6H_4; 9H of **26b**, C_6H_5, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.18, 26.31, 28.01, 28.15 ($C(\mathbf{C}H_3)_2$, **26a/b**), 28.96, 29.05 (C-2, **26a/b**), 32.44, 32.52 (C-6, **26a/b**), 44.79, 44.87, 44.93 (4C, C-1', **26a/b**; C-2', **26a/b**), 55.49 (OCH_3, **26a/b**), 63.79, 63.90 (C-1, **26a/b**), 71.61, 71.66 (C-3, **26a/b**), 72.53, 72.78 (C-4, **26a/b**), 74.76, 74.95 (C-5, **26a/b**), 108.92, 109.00 ($\mathbf{C}(CH_3)_2$, **26a/b**), 113.76, 125.31, 127.30, 127.94, 127.97, 128.24, 128.55, 129.05, 129.92, 130.41, 142.14, 163.61 (C_6H_5, **26a/b**; C_6H_4, **26a/b**), 195.35 (CO, **26a/b**); Missing signals are due to overlapping or low resolution. IR (NaCl) ν 1601 vs (CO) (cm^{-1}); Anal. calcd for $C_{25}H_{30}O_5S$ + 0.25 H_2O: C 67.17; H 6.82; Found: C 66.95; H 6.54.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-*S*-[(1*RS*)-2-oxo-1,2-diphenyl-ethyl]-6-thio-D-*lyxo*-hexitol (27a/b), (CRV 25a/b) **(a:b 1:1).**

According to the general procedure, **11** (63 mg, 0.26 mmol) was deprotected with hydrazine acetate (29 mg, 0.31 mmol) and reacted with desylchloride (89 mg, 0.38 mmol) for 23 h. After work-up and purification according to the general procedure an additional purification by silica gel chromatography (PE/EA 4:1, +0.5% Et_3N) was done and **27a/b** (67 mg, 66%) was obtained as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30, 1.31, 1.48, 1.50 (4s, 6H of **27a**, $C(CH_3)_2$; 6H of **27b**, $C(CH_3)_2$), 1.68-1.78 (m, 2H of **27a**, H-2; 2H of **27b**), 2.67 (m, 1H of **27a**, H-6a), 2.77-2.87 (m, 2H of

27b, H-6), 2.93 (m, 1H of **27a**, H-6b), 3.25-3.32 (m, 1H of **27a**, H-1a; 1H of **27b**, H-1a), 3.51 (t, $J_{5,6a}$ = $J_{5,6b}$ = 7.0 Hz, 1H of **27b**, H-5), 3.65 (m, 1H of **27a**, H-5), 3.77 (m, 1H of **27a**, H-1b), 3.90 (m, 1H of **27b**, H-1b), 4.02 (d, $J_{3,4}$ = 5.5 Hz, 1H of **27a**, H-4), 4.09 (d, $J_{3,4}$ = 5.5 Hz, 1H of **27b**, H-4), 4.21 (m, 1H of **27a**, H-3; 1H of **27b**, H-3), 7.26-7.33, 7.38-7.41, 7.44-7.52, 7.94-7.98 (m, 10H of **27a**, $2C_6H_5$; 10H of **27b**, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.24, 26.31, 28.01, 28.05 (C(**C**H_3)$_2$, **27a/b**), 28.78 (C-2, **27a/b**), 32.37, 32.96 (C-6, **27a/b**), 56.35, 56.50 (C-1', **27a/b**), 63.51, 63.68 (C-1, **27a/b**), 71.60, 71.63 (C-3, **27a/b**), 72.72, 73.41 (C-4, **27a/b**), 75.14, 76.43 (C-5, **27a/b**), 109.01, 109.04 (**C**$(CH_3)_2$, **27a/b**), 127.92, 128.02, 128.55, 128.58, 128.77, 128.88, 128.92, 128.97, 129.04, 133.15, 133.26, 133.81, 136.91, 137.05 (24C, $2C_6H_5$, **27a/b**), 195.22, 195.41 (CO, **27a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{23}H_{26}NaO_4S$ $[M+Na]^+$: 421.14; Found m/z: 421.06; IR (NaCl) ν 1683 vs (CO) (cm^{-1}); Anal. calcd for $C_{23}H_{26}O_4S$: C 69.32, H 6.58; Found : C 69.13, H 6.79.

1,5-Anhydro-2-deoxy-6-*S*-[(1*RS*)-3-oxo-cyclohexyl]-6-thio-D-*lyxo*-hexitol (28a/b), (CRV 8a/b) **(a:b 1:1).**

16a/b (28.9 mg, 0.096 mmol) was stirred overnight at 95°C in 80% aqueous AcOH (2.5 mL). After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **28a/b** (18 mg, 72%) as a white powder after a final lyophilization from dioxane/H_2O (1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.68-1.80 (m, 4H of **28a**, H-2, 2H of C_6H_9; 4H of **28b**, H-2, 2H of C_6H_9), 2.06 (d, $J_{4,OH}$ = 7.1 Hz, 1H of **28a**, OH-4; 1H of **28b**, OH-4), 2.10-2.22, 2.29-2.45, 2.70-2.79 (m, 8H of **28a**, OH-3, H-6a, 6H of C_6H_9; 8H of **28b**, OH-3, H-6a, 6H of C_6H_9), 2.87 (m, 1H of **28a**, H-6b; 1H of **28b**, H-6b), 3.16 (m, 1H of **28a**, H-1'; 1H of **28b**, H-1'), 3.34-3.44 (m, 2H of **28a**, H-1a, H-5; 2H of **28b**, H-1a, H-5), 3.71 (m, 1H of **28a**, H-3; 1H of **28b**, H-3), 3.87 (m, 1H of **28a**, H-4; 1H of **28b**, H-4), 4.00 (m, 1H of **28a**, H-1b; 1H of **28b**, H-1b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.51 (CH_2, **28a/b**), 29.78, 30.11 (C-2, **28a/b**), 31.58, 31.78, 32.00 (C-6, CH_2, **28a/b**), 41.31 (CH_2, **28a/b**), 43.97 (C-1', **28a/b**), 48.27, 48.63 (CH_2, **28a/b**), 66.52 (C-1, **28a/b**), 69.57, 69.76 (4C, C-3, C-4, **28a/b**), 78.96, 79.15 (C-5, **28a/b**), 209.38 (CO, **28a/b**); Missing signals are due to overlapping or low resolution. Anal. calcd for $C_{12}H_{20}O_4S$: C 55.36; H 7.74; Found: C 55.54; H 8.03.

1,5-Anhydro-2-deoxy-6-*S*-[(1*RS*)-2-oxo-cyclohexyl]-6-thio-D-*lyxo*-hexitol (29a/b), (CRV 16a/b), **(a:b 1:1).**

18a/b (130 mg, 0.434 mmol) was stirred at 90°C in 80% aqueous AcOH (19 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **29a/b** (66 mg, 59%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.65-2.02, 2.14-2.26, 2.62-2.95 (m, 14H of **29a**, H-2, H-6, OH-3, OH-4, 8H of C_6H_9; 14H of **29b**, H-2, H-6, OH-3, OH-4, 8H of C_6H_9), 3.32-3.43 (m, 3H of **29a**, H-1a, H-5, H-1'; 3H of **29b**, H-1a, H-5, H-1'), 3.68 (m, 1H of **29a**, H-3; 1H of **29b**, H-3), 3.82 (m, 1H of **29a**, H-4; 1H of **29b**, H-4), 3.97 (m, 1H of **29a**, H1b; 1H of **29b**, H1b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.90, 22.00 (CH_2 of C_6H_9, **29a/b**), 26.91 (C-2, **29a/b**), 29.33, 29.36, 32.26, 32.64, 33.08, 33.23, 37.96, 38.03 (C-6, **29a/b**; CH_2 of C_6H_9, **29a/b**), 52.46, 52.85 (C-1', **29a/b**), 66.11 (C-1, **29a/b**), 69.19, 69.34, 69.37 (C-3, **29a/b**; C-4, **29a/b**), 77.93, 78.18 (C-5, **29b**), 208.78, 208.85 (CO, **29a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{12}H_{20}NaO_4S$ $[M+Na]^+$: 283.10; Found m/z: 283.06; IR (NaCl) ν 1699 vs (CO) (cm^{-1}); Anal. calcd for $C_{12}H_{20}O_4S$ + 0.25 H_2O: C 54.42; H 7.80; Found: C 54.56; H 7.66.

1,5-Anhydro-2-deoxy-6-*S*-[(1*RS*)-2-oxo-cyclopentyl]-6-thio-D-*lyxo*-hexitol (30a/b), (CRV 12a/b), **(a:b 1:1).**

19a/b (114 mg, 0.397 mmol) was stirred at 90°C in 80% aqueous AcOH (18 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) to obtain **30a/b** (83 mg, 85%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.72-1.96, 2.03-2.47, 2.67 (m, 8H of **30a**, H-2, 6H of C_5H_7; 8H of **30b**, H-2, 6H of C_5H_7), 2.75-2.88 (m, 1H of **30a**, H-6a; 2H of **30b**, H-6), 3.07 (m, 1H of **30a**, H-6b), 3.21 (m, 1H of **30a**, H-1'; 1H of **30b**, H-1'), 3.36-3.47 (m, 2H of **30a**, H-1a, H-5; 2H of **30b**, H-1a, H-5), 3.69 (m, 1H of **30a**, H-3; 1H of **30b**, H-3), 3.86 (m, 1H of **30a**, H-4; 1H of **30b**, H-4), 3.98 (m, 1H of **30a**, H1b; 1H of **30b**, H1b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.34 (CH_2 of C_5H_7, **30a/b**), 29.31, 29.32 (C-2, **30a/b**), 30.23, 30.27 (CH_2 of C_5H_7, **30a/b**), 31.64, 31.91 (C-6, **30a/b**), 36.09 (CH_2 of C_5H_7, **30a/b**), 47.58, 48.04 (C-1', **30a/b**), 66.10, 66.18 (C-1, **30a/b**), 69.19, 69.35, 69.38 (4C, C-3, C-4, **30a/b**), 77.94, 78.64 (C-5, **30a/b**), 214.61,

215.14 (CO, **30a/b**), Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{11}H_{18}NaO_4S$ $[M+Na]^+$: 269.08; Found m/z: 269.00; IR (NaCl) ν 1728 vs (CO) (cm^{-1}); Anal. calcd for $C_{11}H_{18}O_4S$: C 53.64; H 7.37; Found : C 53.73; H 7.35.

1,5-Anhydro-2-deoxy-6-*S*-[2-oxo-2phenyl-ethyl]-6-thio-D-*lyxo*-hexitol (31), (CRV 20).

20 (106 mg, 0.329 mmol) was stirred at 95°C in 80% aqueous AcOH (15 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **31** (89 mg, 95%) as white powder after a final lyophilization from dioxane. $[\alpha]_D^{21}$ +3.7 (*c* = 0.56, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.71-1.83 (m, 2H, H-2), 2.43 (d, $J_{3,OH}$ = 7.5 Hz, 1H, OH-3), 2.60 (d, $J_{4,OH}$ = 6.6 Hz, 1H, OH-4), 2.75 (dd, $J_{5,6a}$ = 6.4 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6a), 2.91 (dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 13.6 Hz, 1H, H-6b), 3.34-3.43 (m, 2H, H-1a, H-5), 3.68 (m, 1H, H-3), 3.86-3.95 (m, 4H, H-1b, H-4, H-1'), 7.47, 7.58, 7.96 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.41 (C-2), 33.11 (C-6), 38.10 (C-1'), 66.06 (C-1), 69.30, 69.37 (C-3, C-4), 78.30 (C-5), 128.76, 133.61, 135.15 (6C, C_6H_5), 194.92 (CO); MS (ESI): Calcd for $C_{14}H_{18}NaO_4S$ $[M+Na]^+$: 305.08; Found m/z: 304.99; IR (NaCl) ν 1668 vs (CO) (cm^{-1}); Anal. calcd for $C_{14}H_{18}O_4S$ + 0.25 H_2O: C 58.62; H 6.50; Found: C 58.92; H 6.38.

1,5-Anhydro-2-deoxy-6-*S*-[2-(4-fluorophenyl)-2-oxo-ethyl]-6-thio-D-*lyxo*-hexitol (32), (CRV 21).

21 (91 mg, 0.27 mmol) was stirred at 95°C in 80% aqueous AcOH (12 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **32** (65 mg, 80%) as white powder after a final lyophilization from dioxane. $[\alpha]_D^{21}$ +1.6 (*c* = 0.34, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.69-1.83 (m, 2H, H-2), 2.39 (s, 1H, OH-3), 2.55 (d, $J_{4,OH}$ = 6.0 Hz, 1H, OH-4), 2.74 (dd, $J_{5,6a}$ = 6.2 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6a), 2.90 (dd, $J_{5,6b}$ = 7.3 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6b), 3.39 (m, 2H, H-1a, H-5), 3.69 (m, 1H, H-3), 3.85 (s, 1H, H-4), 3.88 (s, 2H, H-1'), 3.96 (m, 1H, H-1b), 7.14, 8.00 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.36 (C-2), 33.15 (C-6), 38.01 (C-1'), 66.07 (C-1),

69.31, 69.42 (C-3, C-4), 78.31 (C-5), 115.83, 116.00, 131.46, 131.53 (6C, C_6H_4), 193.37 (CO); MS (ESI): Calcd for $C_{14}H_{17}FO_4NaS$ $[M+Na]^+$:323.07; Found: 323.01; IR (NaCl) ν 1674 vs (CO) (cm^{-1}); Anal. calcd for $C_{14}H_{17}FO_4S$: C 55.99; H 5.70; Found : C 56.01; H 5.77.

1,5-Anhydro-2-deoxy-6-*S*-[2-(4-chlorophenyl)-2-oxo-ethyl]-6-thio-D-*lyxo*-hexitol (33), (CRV 22).

22 (83 mg, 0.23 mmol) was stirred at 85°C in 80% aqueous AcOH (10 mL) for 6 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **33** (51 mg, 69%) as white powder after a final lyophilization from dioxane. $[\alpha]_D^{21}$ +0.5 (*c* = 0.35, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.71-1.83 (m, 2H, H-2), 2.70 (m, 3H, H-6a, 2OH), 2.90 (dd, $J_{5,6b}$ = 6.4 Hz, $J_{6a,6b}$ = 12.9 Hz, 1H, H-6b), 3.38 (m, 2H, H-1a, H-5), 3.68 (m, 1H, H-3), 3.83 (s, 1H, H-4), 3.86 (s, 2H, H-1'), 3.92 (m, 1H, H1-b), 7.43, 7.89 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.29 (C-2), 33.19 (C-6), 38.03 (C-1'), 66.06 (C-1), 69.32, 69.46 (C-3, C-4), 78.32 (C-5), 129.08, 130.19, 133.44, 140.07 (6C, C_6H_4Cl), 193.70 (CO); MS (ESI): Calcd for $C_{14}H_{17}ClO_4NaS$ $[M+Na]^+$: 339.04; Found: 338.94; IR (NaCl) ν 1668 vs (CO) (cm^{-1}); Anal. calcd for $C_{14}H_{17}ClO_4S$: C 53.08; H 5.41; Found : C 52.86; H 5.45.

1,5-Anhydro-2-deoxy-6-*S*-[2-(2,5-dimethoxy-phenyl)-2-oxo-ethyl]-6-thio-D-*lyxo*-hexitol (34), (CRV 27).

23 (61 mg, 0.16 mmol) was stirred at 90°C in 80% aqueous AcOH (7 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **34** (42 mg, 78%) as colorless oil. $[\alpha]_D^{21}$ +1.8 (*c* = 0.98, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.75-1.83 (m, 2H, H-2), 2.45 (m, 1H, OH-3), 2.64 (d, $J_{4,OH}$ = 6.1 Hz, 1H, OH-4), 2.71 (dd, $J_{5,6a}$ = 7.0 Hz, $J_{6a,6b}$ = 13.7 Hz, 1H, H-6a), 2.84 (dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 13.7 Hz, 1H, H-6b), 3.38 (m, 2H, H-1a, H-5), 3.67 (m, 1H, H-3), 3.79 (s, 3H, OCH_3), 3.87 (s, 4H, H-4, OCH_3), 3.91-3.98 (m, 3H, H-1b, H-1'), 6.91, 7.06, 7.36 (m, 3H, C_6H_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.50 (C-2), 32.77 (C-6), 43.03 (C-1'), 55.85, 56.16 ($2OCH_3$), 66.10 (C-1),

69.14, 69.33 (C-3, C-4), 78.10 (C-5), 113.24, 114.50, 121.27, 125.86, 153.41, 153.60 (C_6H_3); MS (ESI): Calcd for $C_{16}H_{22}NaO_6S$ $[M+Na]^+$: 365.10; Found m/z: 365.04; IR (NaCl) ν 1661 vs (CO) (cm^{-1}); Anal. calcd for $C_{16}H_{22}O_6S$: C 56.13; H 6.48; Found : C 56.12; H 6.40.

1,5-Anhydro-2-deoxy-6-*S*-(2-napht-2'yl-2-oxo-ethyl)-6-thio-D-*lyxo*-hexitol (35), (CRV 28).

24 (50 mg, 0.133 mmol) was stirred at 90°C in 80% aqueous AcOH (6 mL) for 6 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) afforded **35** (33 mg, 76%) as white solid. $[\alpha]_D^{21}$ +4.1 (*c* = 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.75-1.83 (m, 2H, H-2), 2.33 (d, $J_{3,OH}$ = 8.3 Hz, 1H, OH-3), 2.55 (d, $J_{4,OH}$ = 6.5 Hz, 1H, OH-4), 2.80 (dd, $J_{5,6a}$ = 6.4 Hz, $J_{6a\ 6b}$ = 13.9 Hz, 1H, H-6a), 2.95 (dd, $J_{5,6b}$ = 7.1 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H, H-6b), 3.38 (m, 1H, H-1a), 3.45 (m, 1H, H-5), 3.69 (m, 1H, H-3), 3.87 (s, 1H, H-4), 3.94 (dd, $J_{1a,1b}$ = 11.8 Hz, $J_{1b,2a}$ = $J_{1b,2b}$ = 4.8 Hz, 1H, H-1b), 4.04 (m, 2H, H-1'), 7.55-7.63, 7.90, 7.97, 8.02, 8.50 (m, 7H, $C_{10}H_7$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.44 (C-2), 33.16 (C-6), 38.22 (C-1'), 66.08 (C-1), 69.22 (C-3, C-4), 78.37 (C-5), 124.21, 126.96, 127.83, 128.68, 128.82, 129.69, 130.68, 132.48, 135.79 (10C, $C_{10}H_7$), 194.93 (CO); MS (ESI): Calcd for $C_{18}H_{20}NaO_4S$ $[M+Na]^+$: 355.10; Found m/z: 355.02; IR (NaCl) ν 1668 vs (CO) (cm^{-1}); Anal. calcd for $C_{18}H_{20}O_4S$: C 65.04; H 6.06; Found : C 65.26; H 6.23.

1,5-Anhydro-2-deoxy-6-*S*-[(2*RS*)-1-oxo-indan-2yl]-6-thio-D-*lyxo*-hexitol (36 a/b), (CRV 30a/b), **(a:b 1:1).**

25a/b (65 mg, 0.19 mmol) was stirred at 95°C in 80% aqueous AcOH (10mL) for 6 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **36a/b** (37 mg, 64%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.66-1.86 (m, 2H of **36a**, H-2; 2H of **36b**, H-2), 2.41 (m, 1H of **36a**, OH-3; 1H of **36b**, OH-3), 2.68, 2.78 (d, $J_{4,OH}$ = 6.1 Hz, 1H of **36a**, OH-4; 1H of **36b**, OH-4), 2.90-2.99 (m, 2H of **36a**, H-6a, H-3'a; 2H of **36b**, H-6a, H-3'a), 3.07 (dd, $J_{5,6b}$ = 6.4 Hz, $J_{6a,6b}$ = 13.7 Hz, 1H of **36a**, H-6b), 3.25 (dd, $J_{5,6b}$ = 6.5 Hz, $J_{6a,6b}$ = 13.4 Hz, 1H of **36b**, H-6b), 3.35-3.48 (m, 1H of **36a**, H-1a; 2H of **36b**, H-1a,

H-5), 3.54 (m, 1H of **36a**, H-5), 3.58-3.64 (m, 1H of **36a**, H-3'b; 1H of **36b**, H-3'b), 3.71-3.77 (m, 2H of **36a**, H-3, H-2'; 2H of **36b**, H-3, H-2'), 3.89-4.00 (m, 2H of **36a**, H-1b, H-4; 2H of **36b**, H-1b, H-4), 7.38-7.44, 7.62, 7.78 (m, 4H of **36a**, C_6H_4; 4H of **36b**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.48 (C-2, **36a/b**), 31.36, 32.05 (C-6, **36a/b**), 34.28, 34.57 (C-3', **36a/b**), 45.72, 46.34 (C-2', **36a/b**), 65.99, 66.14 (C-1, **36a/b**), 68.94, 69.25, 69.31, 69.37 (C-3, **36a/b**; C-4, **36a/b**), 78.20,78.61 (C-5, **36a/b**), 124.72,124.79, 126.36, 126.41, 127.04,127.94, 127.99, 135.05, 135.43, 152.08 (12C, C_6H_4, **36a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{15}H_{18}NaO_4S$ $[M+Na]^+$: 317.08; Found m/z: 317.06; IR (NaCl) ν 1694 vs (CO) (cm^{-1}) Anal. calcd for $C_{15}H_{18}O_4S$ + 0.25 H_2O: C 60.28; H 6.23; Found : C 60.27; H 6.18.

1,5-Anhydro-2-deoxy-6-*S*-[(1*RS*)-3-(4-methoxy-phenyl)-3-oxo-1-phenyl-propyl]-6-thio-D-*lyxo*-hexitol (37a/b), (CRV 13a/b), **(a:b 1:1).**

26a/b (129 mg, 0.293 mmol) was stirred at 80°C in 80% aqueous AcOH (13 mL) for 3 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **37a/b** (99.5 mg, 85%) as a white solid. ^1H NMR (500 MHz, CD_3OD): δ 0.98, 1.24-1.34 (m, 2H of **37a**, H-2; 2H of **37b**, H-2), 2.00-2.11 (m, 2H of **37a**, H-6; 2H of **37b**, H-6), 2.51 (t, 1H of **37a**, $J_{5,6a} = J_{5,6b} = 6.9$ Hz, H-5), 2.63 (t, 1H of **37b**, $J_{5,6a} = J_{5,6b} = 6.9$ Hz, H-5), 2.69-2.82 (m, 1H of **37a**, H-1a; 1H of **37b**, H-1a), 2.93-3.04 (m, 3H of **37a**, H-3, H-2'; 3H of **37b**, H-3, H-2'), 3.17 (m, 1H of **37a**, H-4; 1H of **37b**, H-4), 3.27-3.35 (m, 1H of **37a**, H-1b; 1H of **37b**, H-1b), 4.01 (m, 1H of **37a**, H-1'; 1H of **37b**, H-1'), 4.33 (s, 3H of **37a**, OCH_3; 3H of **37b**, OCH_3), 6.43, 6.66, 6.75, 6.88, 7.38 (m, 9H of **37a**, C_6H_5, C_6H_4; 9H of **37b**, C_6H_5, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 30.16, 30.20 (C-2, **37a/b**), 33.32, 33.89 (C-6, **37a/b**), 46.05, 46.12 (C-2', **37a/b**), 46.83, 47.30 (C-1', **37a/b**), 56.49 (OCH_3, **37a/b**), 67.48 (C-1, **37a/b**), 70.42, 70.49 (C-4, **37a/b**), 71.24 (C-3, **37a/b**), 79.95, 80.17 (C-5, **37a/b**), 115.33, 128.72, 129.57, 129.58, 129.92, 131.54, 132.13, 144.05, 144.35, 165.82 (24C, C_6H_5, **37a/b**; C_6H_4, **37a/b**), 198.38 (CO, **37a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{22}H_{26}NaO_5S$ $[M+Na]^+$: 425.14; Found m/z: 425.14; IR (NaCl) ν 1599 vs (CO) (cm^{-1}); Anal. calcd for $C_{22}H_{26}O_5S$ + 0.25 H_2O: C 64.92; H 6.56; Found: C 64.93; H 6.41.

1,5-Anhydro-2-deoxy-6-*S*-[(1*RS*)-2-oxo-1,2-diphenyl-ethyl]-6-thio-D-*lyxo*-hexitol (38a/b), (CRV 29a/b), **(a:b 1:1).**

27a/b (39 mg, 0.099 mmol) was stirred at 90°C in 80% aqueous AcOH (5 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 20:1 b) Hexane c) DCM/MeOH 20:1], followed by filtration through Gelman Acrodisc (0.2 μm) yielded **38a/b** (23 mg, 66%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.71-1.78 (m, 2H of **38a**, H-2; 2H of **38b**, H-2), 2.23 (m, 2H of **38a**, OH-3, OH-4; 1H of **38b**, OH-3**),** 2.52 (d, $J_{4,OH}$ = 6.2 Hz, 1H of **38b**, OH-4), 2.65 (m, 1H of **38a**, H-6a), 2.74 (m, 1H of **38b**, H-6a), 2.81-2.89 (m, 1H of **38a**, H-6b; 1H of **38b**, H-6b), 3.22-3.36 (m, 2H of **38a**, H-1a, H-5; 2H of **38b**, H-1a, H-5), 3.62 (m, 1H of **38a**, H-3; 1H of **38b**, H-3), 3.80-3.92 (m, 2H of **38a**, H-1b, H-4; 2H of **38b**, H-1b, H-4), 7.26-7.53, 7.96 (m, 10H of **38a**, $2C_6H_5$; 10H of **38b**, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.33, 29.39 (C-2, **38a/b**), 32.10, 32.51 (C-6, **38a/b**), 56.23, 56.49 (C-1', **38a/b**), 65.89, 66.04 (C-1, **38a/b**), 69.19, 69.21, 69.28, 69.79 (C-3, **38a/b**; C-4, **38a/b**), 78.15, 79.26 (C-5, **38a/b**), 118.72, 128.07, 128.14, 128.63, 128.67, 128.81, 128.97, 129.05, 133.32, 133.41, 135.57, 135.62, 136.71 (24C, $4C_6H_5$, **38a/b**), 195.28, 195.41 (CO, **38a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{20}H_{22}NaO_4S$ $[M+Na]^+$: 381.11; Found m/z: 381.06; IR (NaCl) ν 1668 vs (CO) (cm^{-1}); Anal. calcd for $C_{20}H_{22}O_4S$ + 0.25 H_2O: C 65.37; H 6.31; Found : C 65.61; H 6.54.

Bis [1,5-anhydro-D-*lyxo*-hexitol-6-yl]-disulphide (39), (CRV 31).

15 (278 mg, 0.683 mmol) was stirred at 80°C in 80% aqueous AcOH (30 mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 9:1 b) Hexane c) DCM/MeOH 9:1], followed by filtration through Gelman Acrodisc (0.2 μm) yielded **39** (156 mg, 70%) as a white solid. $[\alpha]_D^{21}$ +9.4 (*c* = 0.60, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.60 (m, 1H, H-2a), 1.88 (m, 1H, H-2b), 2.94 (m, 2H, H-6), 3.45 (m, 1H, H-1a), 3.54 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, 1H, H-5), 3.69 (m, 1H, H-3), 3.77 (s, 1H, H-4), 3.95 (m, 1H, H-1b); ^{13}C NMR (125 MHz, CD_3OD): δ 30.20 (C-2), 41.82 (C-6), 67.50 (C-1), 70.80, 71.21 (C-3, C-4), 79.14 (C-5); MS (ESI): Calcd for $C_{12}H_{22}O_6NaS_2$ $[M+Na]^+$: 349.08; Found m/z: 348.96; Anal. calcd for $C_{12}H_{22}O_6S_2$: C 44.16; H 6.79; Found : C 44.17; H 6.87.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-{4-(napht-2-yl)-[1,2,3]-triazol-1-yl}-D-*lyxo*-hexitol (42), (CRVII 9).

41 (20 mg, 0.094 mmol) was dissolved in H_2O/t-BuOH (1:1) (1.7 mL) with vigorous stirring. 1-Ethynyl-naphtalene (13.7 μL, 0.094 mmol) was added followed by sodium ascorbate as a freshly prepared solution (93 μL, 20 mg/mL in H_2O/t-BuOH 1:1) and finally $CuSO_4 \cdot 5H_2O$ also as a freshly prepared solution (23 μL, 10 mg/mL in H_2O). The reaction was stirred at r.t. Additions of fresh sodium ascorbate and copper sulfate solutions (equal amounts to the initial one) were performed every 4 to 6 h during 4 days. When no more changes were detected according to TLC, one eq of alkyne was added (this was repeated twice). After 4 days the reaction was concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1, +1% Et_3N) gave **42** (31 mg, 91%) as white solid. $[\alpha]_D^{21}$ -10.0 (*c* = 0.91, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.38, 1.58 (2s, 6H, $C(CH_3)_2$), 1.79-1.92 (m, 2H, H-2), 3.41 (m, 1H, H-1a), 3.98 (m, 1H, H-1b), 4.09 (m, 2H, H-4, H-5), 4.33 (m, 1H, H-3), 4.66 (dd, $J_{5,6a}$ = 8.5 Hz, $J_{6a,6b}$ = 14.2 Hz, 1H, H-6a), 4.78 (dd, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 14.2 Hz, 1H, H-6b), 7.52, 7.75, 7.89, 8.40 (m, 8H, C_2HN_3, $C_{10}H_7$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.16, 27.93, 28.60 (C-2, $C(\mathbf{C}H_3)_2$), 51.70 (C-6), 63.42 (C-1), 71.44 (C-3), 72.18 (C-4), 73.96 (C-5), 109.53 ($\mathbf{C}(CH_3)_2$), 124.30, 125.37, 125.92, 126.53, 127.13, 128.10, 128.42, 128.77, 131.04, 133.86, 146.49 (12C, C_2HN_3, $C_{10}H_7$); MS (ESI): Calcd for $C_{21}H_{24}N_3O_3$ $[M+H]^+$: 366.18; Found m/z: 366.20; Anal. calcd for $C_{21}H_{23}N_3O_3$ + 0.17 H_2O: C 68.46; H 6.37; N 11.37 Found : C 68.79; H 6.69; N 10.83.

1,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-6-{4-(3-trifluoromethyl-phenyl)-[1,2,3]-triazol-1-yl}-D-*lyxo*-hexitol (43), (CR VII 11).

41 (21 mg, 0.10 mmol) was dissolved in H_2O/t-BuOH (1:1) (1.7 mL) with vigorous stirring. 3-Ethynyl-α,α,α-trifluorotoluene (15 μL, 0.10 mmol) was added followed by sodium ascorbate as a freshly prepared solution (50 μL, 20 mg/mL in H_2O/t-BuOH 1:1) and finally $CuSO_4 \cdot 5H_2O$ also as a freshly prepared solution (25 μL, 10 mg/mL in H_2O). The reaction was stirred at r.t. Identical additions of fresh sodium ascorbate and copper sulfate solutions as the initial one, were performed every 3 h along the whole reaction time. When no more changes were detected according to TLC, one eq of alkyne was added (this was repeated once). After 48 h, a catalytic amount of

CuCl was added and the reaction left overnight. The mixture was concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1, +1% Et_3N) gave **43** (25 mg, 67%) as white solid. $[\alpha]_D^{21}$ +6.6 (*c* = 1.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.37, 1.57 (2s, 6H, $C(CH_3)_2$), 1.80-1.92 (m, 2H, H-2), 3.38 (m, 1H, H-1a), 3.96-4.06 (m, 3H, H-1b, H-4, H-5), 4.32 (m, 1H, H-3), 4.58 (dd, $J_{5,6a}$ = 8.8 Hz, $J_{6a,6b}$ = 14.2 Hz, 1H, H-6a), 4.72 (dd, $J_{5,6b}$ = 3.7 Hz, $J_{6a,6b}$ = 14.2 Hz, 1H, H-6b), 7.55, 7.75, 7.98, 8.02, 8.08 (m, 5H, C_2HN_3, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.29, 28.07 ($C(\mathbf{C}H_3)_2$), 28.70 (C-2), 52.06 (C-6), 63.56 (C-1), 71.60 (C-3), 72.37 (C-4), 74.09 (C-5), 109.73 ($\mathbf{C}(CH_3)_2$), 122.02, 122.61, 129.48, 131.68, 146.34 (9C, C_2HN_3 , $C_7H_4F_3$); MS (ESI): Calcd for $C_{18}H_{21}F_3N_3O_3$ $[M+H]^+$: 384.15; Found m/z: 384.19.

1,5-Anhydro-2-deoxy-6-{4-(napht-2-yl)-[1,2,3]-triazol-1-yl}-D-*lyxo*-hexitol (44), (CRVII 10).

42 (25 mg, 0.68 mmol) was stirred at 95°C in 80% aqueous AcOH (3mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 9:1 b) Hexane c) DCM/MeOH 9:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **44** (19 mg, 95%) as white powder after a final lyophilization from H_2O/dioxane (1:1). $[\alpha]_D^{21}$ +11.5 (*c* = 0.95, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 1.62 (m, 1H, H-2a), 1.98 (m, 1H, H-2b), 3.42 (t, $J_{1a,2a}$ = $J_{1b,2b}$ = 12.0 Hz, 1H, H-1a), 3.73 (m, 1H, H-3), 3.79 (m, 1H, H-4), 3.86 (t, $J_{5,6a}$ = $J_{5,6b}$ = 4.1 Hz, 1H, H-5), 3.97 (m, 1H, H-1b), 4.68-4.76 (m, 2H, H-6), 7.54, 7.68, 7.93, 8.24 (m, 8H, C_2HN_3, $C_{10}H_7$); ^{13}C NMR (125 MHz, CD_3OD): δ 29.74 (C-2), 53.23 (C-6), 66.98 (C-1), 69.93 (C-4), 70.36 (C-3), 78.74 (C-5), 126.10, 126.17, 126.21, 126.40, 127.14, 128.37, 129.08, 129.54, 130.10, 132.46, 135.39, 147.38 (C_2HN_3 , $C_{10}H_7$); MS (ESI): Calcd for $C_{18}H_{20}N_3O_3$ $[M+H]^+$: 326.15; Found m/z: 326.15; Anal. calcd for $C_{18}H_{19}N_3O_3$ + 0.17 H_2O: C 65.84; H 6.04; Found : C 66.16; H 6.45.

1,5-Anhydro-2-deoxy-6-{4-(3-trifluoromethyl-phenyl)-[1,2,3]-triazol-1-yl}-D-*lyxo*-hexitol (45), (CRVII 12).

43 (25 mg, 0.065 mmol) was stirred at 80°C in 80% aqueous AcOH (3mL) for 4 h. After cooling to r.t., the solvent was coevaporated with toluene. Purification by silica gel chromatography [a) DCM/MeOH 9:1 b) Hexane c) DCM/MeOH 9:1], followed by filtration through Gelman Acrodisc (0.2 μm) gave **45** (22 mg, 100%) as white powder

after a final lyophilization from H_2O/dioxane (1:1). $[\alpha]_D^{21}$ +6.1 (*c* = 1.10, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 1.61 (m, 1H, H-2a), 1.97 (m, 1H, H-2b), 3.38 (m, 1H, H-1a), 3.73 (m, 1H, H-3, H-4), 3.80 (m, 1H, H-5), 3.96 (m, 1H, H-1b), 4.61-4.70 (m, 2H, H-6), 7.63, 8.08, 8.14 (m, 4H, C_6H_4), 8.43 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 29.73 (C-2), 53.31 (C-6), 66.97 (C-1), 69.91 (C-4), 70.37 (C-3), 78.68 (C-5), 123.13, 130.13, 132.98, 147.11 (9C, C_2HN_3 , $C_7H_4F_3$); MS (ESI): Calcd for $C_{15}H_{17}F_3N_3O_3$ $[M+H]^+$: 344.12; Found m/z: 344.12; Anal. calcd for $C_{15}H_{16}$ $F_3N_3O_3$ + 0.1 H_2O: C 52.20; H 4.73; Found : C 52.49; H 4.91.

3,4-*O*-[(1RS)-4-(Allyl carboxylate)-benzylidene]-1,5-anhydro-6-azido-2-deoxy-6-{4-(tiophen-3-yl)- [1,2,3]-triazol-1-yl}-D-*lyxo*-hexitol (48a/b), (CR VII 5a/b), **(a:b: 1:1).**

47a/b (25 mg, 0.073 mmol) was dissolved in H_2O/t-BuOH (1:1) (1.35 mL) with vigorous stirring. 3-Ethynyl-tiophene (72 μL, 0.073 mmol) was added followed by sodium ascorbate as a freshly prepared solution (144 μL, 10 mg/mL in H_2O/t-BuOH 1:1) and finally $CuSO_4{\cdot}5H_2O$ also as a freshly prepared solution (18.2 μL, 10 mg/mL in H_2O). The reaction was stirred at r.t. and three additional portions of freshly prepared sodium ascorbate and copper sulfate solutions (same quantities as the initial addition) were added during a period of 40 h. The reaction was concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1 to 1:1, +1% Et_3N) gave **48a/b** 1:1 (26.1 mg, 79%) as colorless oil. The two isomers could be separated. **48a**: $[\alpha]_D^{21}$ +31.2 (*c* = 0.34, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.90-2.04 (m, 2H, H-2), 3.38 (dd, $J_{1a,1b}$ = 11.3 Hz, $J_{1a,2a}$ = $J_{1a,2b}$ = 3.1 Hz, 1H, H-1a) 3.99 (d, $J_{3,4}$ = 5.1 Hz, 1H, H-4), 4.05 (m, 2H, H-1b, H-5), 4.57-4.66 (m, 2H, H-3, H-6a), 4.72 (dd, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 14.2 Hz, 1H, H-6b), 4.84 (m, 2H, $CH_2CHC\mathbf{H_2}O$), 5.30 (m, 1H, Ha of $C\mathbf{H_2}CHCH_2O$), 5.42 (m, 1H, Hb of $C\mathbf{H_2}CHCH_2O$), 6.01-6.09 (m, 1H, $CH_2C\mathbf{H}CH_2O$), 6.26 (s, 1H, CHO_2), 7.38 (m, 2H, C_4H_3S), 7.52 (m, 2H, C_6H_4), 7.63 (m, 1H, C_4H_3S), 7.74 (s, 1H, C_2HN_3), 8.08 (m, 2H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.60 (C-2), 51.64 (C-6), 63.95 (C-1), 65.71 ($CH_2CH\mathbf{C}H_2O$), 72.21 (C-4), 72.88 (C-3), 74.77 (C-5), 102.41 (CHO_2), 118.39 ($\mathbf{C}H_2CHCH_2O$), 120.99, 121.19, 125.77, 126.16, 126.32, 129.91, 130.98, 143.56 (12C, C_6H_4, C_4H_3S, C_2HN_3), 132.12 ($CH_2\mathbf{C}HCH_2O$), 165.80 (CO); IR (NaCl) ν 1716 vs (CO) (cm^{-1}). **48b**: $[\alpha]_D^{21}$ -6.8 (*c* = 0.96, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.86 (m, 1H, H-2a), 1.96 (m, 1H, H-2b), 3.50 (m 1H, H-1a)

4.01 (m, 1H, H-1b), 4.12 (m, 1H, H-5), 4.17 (d, $J_{3,4}$ = 6.3 Hz, 1H, H-4), 4.50 (m, 1H, H-3), 4.58 (dd, $J_{5,6a}$ = 8.6 Hz, $J_{6a,6b}$ = 14.3 Hz, 1H, H-6a), 4.76 (dd, $J_{5,6b}$ = 3.7 Hz, $J_{6a,6b}$ = 14.3 Hz, 1H, H-6b), 4.84 (m, 2H, CH_2CHCH_2O), 5.30 (m, 1H, Ha of CH_2CHCH_2O), 5.42 (m, 1H, Hb of CH_2CHCH_2O), 5.95 (s, 1H, CHO_2), 6.01-6.09 (m, 1H, CH_2CHCH_2O), 7.38, 7.46 (m, 2H, C_4H_3S), 7.64 (m, 2H, C_6H_4), 7.68 (m, 1H, C_4H_3S), 7.79 (s, 1H, C_2HN_3), 8.11 (m, 2H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.79 (C-2), 51.66 (C-6), 63.03 (C-1), 65.69 (CH_2CHCH_2O), 71.90 (C-3), 73.54 (C-4), 74.25 (C-5), 103.40 (CHO_2), 118.33 (CH_2CHCH_2O), 121.03, 121.26, 125.83, 126.31, 126.76, 129.83, 131.15, 131.85, 142.24, 143.78 (12C, C_6H_4, C_4H_3S, C_2HN_3), 132.13 (CH_2CHCH_2O), 165.86 (CO); MS (ESI): Calcd for $C_{23}H_{24}N_3O_5S$ $[M+H]^+$: 454.14; Found m/z: 454.15; IR (NaCl) ν 1715 vs (CO) (cm^{-1})

Synthesis on solid phase of:

1,5-Anhydro-2-deoxy-6-{4-(tiophen-3-yl)-[1,2,3]-triazole}-D-*lyxo*-hexitol (50),

(CRVII 8).

Aminomethylated polystyrene lanterns (Mimotopes Synphase^{TM}, D series) with a loading capacity of 35 μmol, were used as solid support.

a) Lantern swelling and activation. The lanterns were immersed for 30 min in DMF, then trested with 20% Et_3N in DMF (3 x 10 min).

b) Standard washing procedure. The lanterns were washed with DMF (3 x 10 min) and DCM (3 x 10 min).

c) Loading. The coupling solution consisted of **49** (69.5 mg, 3.3 eq x 35 μmol x 1.5 lantern) and HOBt (26.2 mg, 3.3 eq x 35 μmol x 1.5 lantern) in DMF (0.75 mL). Of the coupling solution, 0.5 mL was pipetted in a 96-well plate followed by DIC (17.7 μL, 3.3 eq x 35 μmol). After 2 min of activation, the lantern was added and the plate left at r.t. in the shaker during 21 h. The lantern was then washed with the above washing procedure.

d) Triazole formation. To a well containing 0.45 mL of H_2O/*t*-BuOH (1:1), 3-ethynyl-tiophene (11.2 μL, 0.114 mmol) was added followed by freshly prepared sodium ascorbate solution (20.0 μL, 113 mg/mL in H_2O/*t*-BuOH 1:1), and freshly prepared $CuSO_4 \cdot 5H_2O$ solution (10.0 μL, 28.5 mg/ml in H_2O). The lantern was immersed in the previous solution and the plate was left at r.t. in the shaker for 3 days. Regular additions of freshly prepared sodium ascorbate and copper sulfate solutions (equal

amounts as the initial addition) were performed three times per day. After 3 days of reaction, the washing procedure was applied.

d) Cleavage from the solid phase. The lantern was treated with 80% aqueous AcOH containing 2% TFA (2 mL) (2 x 15-20 h). The solvent was evaporated under reduced pressure to provide **50** as a white solid in 58% yield considering the loading capacity of one lantern. ^{1}H NMR (500 MHz, CD_3OD): δ 1.61 (m, 1H, H-2a), 1.95 (m, 1H, H-2b), 3.39 (m, 1H, H-1a), 3.68-3.79 (m, 3H, H-3, H-4, H-5), 3.95 (m, 1H, H-1b), 4.58-4.66 (m, 2H, H-6), 7.49, 7.74 (m, 3H, C_4H_3S), 8.18 (s, 1H, C_2HN_3), ^{13}C NMR (125 MHz, CD_3OD): δ 30.13 (C-2), 53.60 (C-6), 67.36 (C-1), 70.32 (C-4), 70.77 (C-3), 79.14 (C-5), 122.51, 122.49, 127.12, 128.02, 133.25, 145.28 (C_4H_3S, C_2HN_3); MS (ESI): Calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$: 282.09; Found m/z: 281.83.

Synthesis of a library of derivates of methyl α-D-galactopyranoside.

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-α-D-galactopyranoside (51), (CRDM001).

To a suspension of methyl α-D-galactopyranoside (6.24 g, 32.1 mmol) in 120 mL of dry DCM was added TBDPSCl (9.00 mL, 35.3 mmol). DMAP (157 mg, 1.28 mmol) and Et_3N (5.05 mL, 38.5 mmol) were added and the mixture was stirred under argon at r.t.. During the reaction, the pH was controlled and set to basic with Et_3N until no more starting material was detected by TLC. After 64 h the solvent was evaporated and the crude material was redissolved in DCM (30 mL) and washed with water (2 x 20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure. After purification by silica gel chromatography (DCM/MeOH 20:1), **51** (15.1 g, quantitative) was obtained as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.07 (s, 9H, $C(CH_3)_3$), 2.06 (d, $J_{2,OH}$ = 9.4 Hz, 1H, OH-2), 2.60 (d, $J_{3,OH}$ = 5.4 Hz, 1H, OH-3), 2.74 (d, $J_{4,OH}$ = 2.6 Hz, 1H, OH-4), 3.37 (s, 3H, OCH_3), 3.72 (m, 1H, H-3), 3.77 (t, $J_{5,6a}$ = $J_{5,6b}$ = 5.5 Hz, 1H, H-5), 3.82 (m, 1H, H-2), 3.87 (dd, $J_{5,6a}$ = 5.1 Hz, $J_{6a,6b}$ = 10.6 Hz, 1H, H-6a), 3.92 (dd, $J_{5,6b}$ = 6.1 Hz, $J_{6a,6b}$ = 10.6 Hz, 1H, H-6b), 4.12 (s, 1H, H-4), 4.80 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 7.38-7.46, 7.67-7.71 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.56 (**C**$(CH_3)_3$), 27.19 (3C, C(**C**$H_3)_3$), 55.78 (OCH_3), 63.89 (C-6), 69.80 (C-4), 70.14 (C-5), 70.33 (C-2), 71.87 (C-3), 99.79 (C-1), 128.20, 130.30, 133.45, 135.97, 136.05 (12C, $2C_6H_5$).

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-isopropylidene-α-D-galactopyranoside (52), (CRDM002).

Compound **51** (1.09 g, 2.52 mmol) was dissolved under argon in dry CH_3CN (50 mL). 2,2-Dimethoxypropane (0.46 mL, 3.78 mmol) was added dropwise with vigorous stirring followed by toluene-4-sulfonicacid in acetonitrile (1.0 mL, 14.4 mg/mL, 75.6 μmol). After 24 h, 2,2-dimethoxypropane (93.0 μL, 0.756 mmol) was added, and stirring was continued for additional 5 h. After neutralizing the reaction with Et_3N, the solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA 2:1, +0.5% Et_3N) to afford **52** (1.07 g, 89%) as yellow oil. $[\alpha]_D^{21}$ +63.2 (*c* = 0.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H, $C(CH_3)_3$), 1.34, 1.48 (2s, 6H, $C(CH_3)_2$), 2.31 (d, $J_{2,OH}$ = 6.9 Hz, 1H, OH-2), 3.42 (s, 3H, OCH_3), 3.78 (m, 1H, H-2), 3.86 (dd, $J_{5,6a}$ = 6.6 Hz, $J_{6a,6b}$ = 10.1 Hz, 1H, H-6a), 3.93 (dd, $J_{5,6b}$ = 6.6 Hz, $J_{6a,6b}$ = 10.1 Hz, 1H, H-6b), 4.06 (td, $J_{4,5}$ = 2.2 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, 1H, H-5), 4.19 (m, 1H, H-3), 4.28 (m, 1H, H-4), 4.73 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 7.36-7.45, 7.71 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.60 (**C**$(CH_3)_3$), 26.37 (C(**C**$H_3)_2$), 27.17 (3C, C(**C**$H_3)_3$), 28.25 (C(**C**$H_3)_2$), 55.75 (OCH_3), 63.36 (C-6), 68.89 (C-5), 70.27 (C-2), 73.25 (C-4), 76.67 (C-3), 98.92 (C-1), 109.81 (**C**$(CH_3)_2$), 128.02, 128.07, 130.07, 133.82, 133.92, 136.03, 136.06 (12C, $2C_6H_5$).

(2*RS*)-2-(2-Bromoethoxy)-tetrahydro-2*H*-pyran (53), (CRDM006).

To a stirred mixture of 2-bromoethanol (1.60 mL, 21.4 mmol) and 3,4-dihydro-2*H*-pyran (3.00 mL, 32.2 mmol) in dry DCM (115 mL) pyridinium *p*-toluenesulfonate was added (0.54 g, 2.14 mmol) under argon. After stirring at r.t. for 2 h, the solution was washed with water (2 x 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure at rt. The residue was purified by silica gel chromatography (PE/EA 10:1) and **53** was obtained (4.60 g, quantitative) as colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52-1.89 (m, 6H, H-3, H-4, H-5), 3.49-3.55 (m, 3H, H-2', H-6a), 3.77 (dt, $J_{1'a,1'b}$ = 11.3 Hz, $J_{1'a,2'a}$ = $J_{1'a,\ 2'b}$ = 6.3 Hz, 1H, H-1'a), 3.90 (m, 1H, H-6b), 4.02 (dt, $J_{1'a,1'b}$ = 11.3 Hz, $J_{1'b,2'a}$ = $J_{1'b,2'b}$ = 6.3 Hz, 1H, H-1'b), 4.68 (t, *J* = 3.5 Hz, 1H, H-2), ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.65, 25.75, 30.82 (C-3, C-4, C-5), 31.26 (C-2'), 62.68 (C-6), 67.93 (C-1'), 99.34 (C-2).

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-[(2*RS*)-(2-ethoxy-tetrahydro-2*H*-pyran)-2'-*O*-yl]-3,4-*O*-isopropylidene-α-D-galactopyranoside (54a/b), (CRDM007a/b) **(a:b 1:1).**

NaH (313 mg of 55% NaH in oil, 7.20 mmol) was washed with hexane (3 x 10 mL) under argon and immediately dried in high vacuum. After suspending the NaH in dry DMF (30 mL), **52** (1.45 g, 2.87 mmol) dissolved in DMF (10 mL) was slowly added over 30 min at 0°C. Then a solution of **53** (900 mg, 4.31 mmol) in DMF (3 mL) was added during 15 min. After 1 h at 0°C, the reaction was warmed to r.t.. When no more gas evolution was detected, the reaction mixture was heated to 40°C. After 29 h the reaction was quenched by addition of saturated aqueous NH_4Cl until pH 7, extracted with DCM (2 x 40 mL) and washed with water (2 x 30 mL). After drying with Na_2SO_4 and filtering, the organic layer was concentrated under reduced pressure and the residue purified by silica gel chromatography (PE/EA 4:1 to 2:1, +0.5% Et_3N). **54** (620 mg, 50%) was afforded as an isomeric mixture. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H of **54a**, $C(CH_3)_3$; 9H of **54b**, $C(CH_3)_3$), 1.34 (s, 3H of **54a**, $C(CH_3)_2$; 3H of **54b**, $C(CH_3)_2$), 1.52 (s, 5H of **54a**, $C(CH_3)_2$, 2H of C_5H_9O; 5H of **54b**, $C(CH_3)_2$, 2H of C_5H_9O), 1.55-1.62, 1.68-1.74, 1.79-1.83 (m, 4H of **54a**, 4H of C_5H_9O; 4H of **54b**, 4H of C_5H_9O), 3.37 (s, 3H of **54a**, OCH_3; 3H of **54b**, OCH_3), 3.46 (m, 1H of **54a**, 1H of C_5H_9O; 1H of **54b**, 1H of C_5H_9O), 3.55 (m, 1H of **54a**, H-2; 1H of **54b**, H-2), 3.60-3.67, 3.80-3.98 (m, 7H of **54a**, H-6, H-1', H-2', 1H of C_5H_9O; 7H of **54b**, H-6, H-1', H-2', 1H of C_5H_9O), 4.04 (t, $J_{5,6a} = J_{5,6b} = 6.6$ Hz, 1H of **54a**, H-5; 1H of **54b**, H-5), 4.25-4.28 (m, 2H of **54a**, H-3, H-4; 2H of **54b**, H-3, H-4), 4.64 (m, 1H of **54a**, CHO of C_5H_9O; 1H of **54b**, CHO of C_5H_9O), 4.79 (d, $J_{1,2} = 3.5$ Hz, 1H of **54a**, H-1; 1H of **54b**, H-1), 7.36-7.44, 7.70-7.72 (m, 10H of **54a**, $2C_6H_5$; 10H of **54b**, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.59, 19.95 (**C**$(CH_3)_3$, **54a/b**), 25.83 (CH_2 of C_5H_9O, **54a/b**), 26.75, 26.78 (C(**C**$H_3)_2$, **54a/b**), 27.15 (C(**C**$H_3)_3$, **54a/b**), 28.73 (C(**C**$H_3)_2$, **54a/b**), 30.09 (CH_2 of C_5H_9O, **54a/b**), 30.94, 31.01 ($2CH_2$ of C_5H_9O, **54a/b**), 55.77 (OCH_3, **54a/b**), 62.48, 62.71, 63.50, 67.09, 67.41 (OCH_2, **54a/b**; CH_2 of C_5H_9O, **54a/b**), 68.12 (C-5, **54a/b**), 70.61, 71.02 (C-6, **54a/b**), 76.51, 76.42, 73.67 (4C, C-3, **54a/b**; C-4, **54a/b**), 79.06, 78.90 (C-2, **54a/b**), 99.15, 99.55, 98.74, 98.78 (4C, C-1, **54a/b**; CH of C_5H_9O, **54a/b**), 109.39 (**C**$(CH_3)_2$), 128.02, 128.07, 130.08, 131.09, 133.94, 136.07 (24C, $2C_6H_5$, **54a/b**); Missing signals are due to overlaping or low resolution. Anal. calcd for $C_{33}H_{48}O_8Si + 0.25\ H_2O$: C 65.46, H 8.08; Found: C 65.14, H 8.05.

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-(2-hydroxyethyl)-3,4-*O*-isopropylidene-α-D-galactopyranoside (55), (CRDM008).

Dry **54a/b** (1.66 g, 2.76 mmol) was dissolved under argon in MeOH (22 mL) and PPTS (251 mg, 0.138 mmol) was added. The mixture was stirred at 55°C for 5 h and then left at r.t. overnight. The reaction was neutralized by addition of Et_3N (pH 7). The solvents were evaporated and the residue was purified by silica gel chromatography (PE/EA 2:1to 4:3, +0.5% Et_3N) to obtain **55** (83%) as yellowish oil. $[\alpha]_D^{21}$ +58.7 (c = 1.05, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H, $C(CH_3)_3$), 1.34, 1.52 (2s, 6H, $C(CH_3)_2$), 3.38 (s, 3H, OCH_3), 3.50 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 7.6 Hz, 1H, H-2), 3.72-3.81 (m, 4H, H-1', H-2'), 3.87 (dd, $J_{5,6a}$ = 6.5 Hz, $J_{6a,6b}$ = 9.9 Hz, 1H, H-6a), 3.95 (dd, $J_{5,6b}$ = 6.9 Hz, $J_{6a,6b}$ = 9.9 Hz, 1H, H-6b), 4.25-4.31 (m, 2H, H-3, H-4), 4.77 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 7.36-7.45, 7.69-7.74 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.60 (**C**$(CH_3)_3$), 26.60 ($C(\mathbf{C}H_3)_2$), 27.16 (3C, $C(\mathbf{C}H_3)_3$), 28.57 ($C(\mathbf{C}H_3)_2$), 55.78 (OCH_3), 62.36 (C-2'), 63.24 (C-6), 68.18 (C-5), 73.09 (C-1'), 73.61 (C-4), 76.04 (C-3), 79.37 (C-2), 98.24 (C-1), 109.69 (**C**$(CH_3)_2$), 128.03, 128.08, 130.10, 133.76, 133.87, 136.01, 136.05 (12C, $2C_6H_5$); Missing signals are due to overlapping or low resolution. Anal. calcd for $C_{28}H_{40}O_7Si$ + 0.25 H_2O: C 64.53, H 7.83; Found: C 64.48, H 7.90.

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-isopropylidene-2-*O*-[(2-methanesulfonyloxy)ethyl]-α-D-galactopyranoside (56), (CRDM009).

Galactoside **55**(1.15 g, 2.22 mmol) was dissolved in pyridine (20 mL). Then mesyl chloride (0.35 mL, 4.52 mmol) was added dropwise at 0°C. After 15 min the reaction was warmed to r.t. and stirring continued for 3 h. The reaction was diluted with EtOAc (30 mL) and extracted with 1 N HCl (2 x 15 mL), saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated. Silica gel chromatography (PE/EA 2:1, +0.5% Et_3N) of the residue gave **56** (1.22 g, 92%) as a white solid. $[\alpha]_D^{21}$ +59.8 (c = 0.58, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H, $C(CH_3)_3$), 1.33, 1.51 (2s, 6H, $C(CH_3)_2$), 3.08 (s, 3H, SCH_3), 3.36 (s, 3H, OCH_3), 3.48 (m, 1H, H-2), 3.85-3.90 (m, 2H, H-6a, 1H of CH_2), 3.94-4.04 (m, 3H, H-5, H-6b, 1H of CH_2), 4.10-4.14 (m, 1H, H-3), 4.23-4.28 (m, 1H, H-4), 4.39 (m, 2H, CH_2), 4.76 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 7.36-7.44, 7.66-7.74 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.60 (**C**$(CH_3)_3$), 26.71 ($C(\mathbf{C}H_3)_2$), 27.16

(3C, C(**C**H_3)$_3$), 28.78 (C(**C**H_3)$_2$), 38.06 (SCH_3), 55.72 (OCH_3), 63.25 (C-6), 68.11 (C-5), 69.16, 69.81 ($2CH_2$), 73.66 (C-4), 76.17 (C-3), 79.42 (C-2), 98.32 (C-1), 109.59 (**C**$(CH_3)_2$), 128.04, 128.10, 130.12, 133.76, 133.86, 136.01, 136.04 (12C, $2C_6H_5$). Anal. calcd for $C_{29}H_{42}O_9SSi$ + 0.5 H_2O: C 57.69, H 7.18; Found: C 57.60, H 6.93.

Methyl 2-*O*-(2-azidoethyl)-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-isopropylidene-α-D-galactopyranoside (57), (CRDM010).

Compound **56** (613 mg, 1.03 mmol) was dissolved under argon in dry DMF (40 mL). Sodium azide (336 mg, 5.16 mmol) was added and the reaction was vigorously stirred under argon at r.t. overnight. Then it was heated to 50°C for 6 h. The reaction mixture was diluted at 0°C with EtOAc and washed with ice cold water (2 x 40 mL) followed by extraction of the aqueous layer with EtOAc (3 x 40 mL). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and the resulting syrup was purified by silica gel chromatography (PE/EA 8:1, +0.5% Et_3N) to afford **57** (558 mg, quantitative) as a white foam. $[\alpha]_D^{21}$ +50.8 (c = 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (s, 9H, C$(CH_3)_3$), 1.34, 1.51 (2s, 6H, C$(CH_3)_2$), 3.29-3.33 (m, 1H, H-2'a), 3.38 (s, 3H, OCH_3), 3.47-3.53 (m, 2H, H-2, H-2'b), 3.76 (m, 1H, H-1'a), 3.87 (dd, $J_{5,6}$ = 6.5 Hz, $J_{6a,6b}$ = 9.9 Hz, 1H, H-6a), 3.96 (m, 2H, H-6b, H-1'b), 4.05 (td, $J_{4,5}$ = 1.4 Hz, $J_{5,6}$ = 6.5 Hz, 1H, H-5), 4.27 (m, 2H, H-3, H-4), 4.77 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 7.36-7.44, 7.70 (m, 10H, $2C_6H_5$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.77 (**C**$(CH_3)_3$), 28.90 (C(**C**H_3)$_2$), 29.33 (3C, C(**C**H_3)$_3$), 30.94 (C(**C**H_3)$_2$), 53.29 (C-2'), 58.03 (OCH_3), 65.47 (C-6), 70.29 (C-5), 72.71 (C-1'), 75.86, 78.59 (C-3, C-4), 81.52 (C-2), 100.77 (C-1), 111.68 (**C**$(CH_3)_2$), 130.20, 130.26, 132.27, 135.97, 136.07, 138.19, 138.22 (12C, $2C_6H_5$); IR (NaCl) ν 2103 s (-N=N$^+$-N$^-$) (cm^{-1}) ; Anal. calcd for $C_{28}H_{39}N_3O_6Si$: C 62.08, H 7.26, N 7.76; Found: C 62.14, H 7.17, N 7.76.

Methyl 2-*O*-(2-azidoethyl)-α-D-galactopyranoside (58), (CRDM015).

Compound **57** (75.4 mg, 0.139 mmol) was stirred overnight at 50°C in 80% aqueous AcOH (6 mL). After cooling to r.t., the solvent was coevaporated four times with toluene. The crude material was dried at high vacuum and used without further purification. It was dissolved in THF (3.7 mL) and 1 M tetrabutylammonium fluoride solution in THF (0.28 mL, 0.28 mmol) was added. The mixture was stirred at r.t. for 2 h. The solvent was evaporated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 20:1) gave **58** (40 mg, quantitative) as colourless oil.

$[\alpha]_D^{21}$ +116.0 (c = 0.45, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.46 (s, 1H, OH-6), 2.97 (s, 1H, OH-3), 3.04 (s, 1H, OH-4), 3.40-3.44 (m, 4H, H-2'a, OCH_3), 3.51-3.56 (m, 1H, H-2'b) 3.71-3.77 (m, 2H, H-2, H-1'a), 3.82-3.93 (m, 3H, H-5, H-6a, H-1'b), 3.96-4.01 (m, 2H, H-3, H-6b), 4.13 (d, $J_{3,4}$ = 2.8 Hz, 1H, H-4), 4.95 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.02 (C-2'), 55.47 (OCH_3), 63.26 (C-6), 69.07, 69.11 (C-3, C-5), 69.79 (C-1'), 70.61 (C-4), 77.98 (C-2), 97.72 (C-1); MS (ESI): Calcd for $C_9H_{17}N_3NaO_6$ $[M+Na]^+$: 286.10; Found m/z: 286.04; IR (NaCl) ν 2100 s ($-N=N^+-N^-$) (cm^{-1}).

General procedure for the synthesis of 1,4-disubstituted-[1,2,3]-triazoles from 57.

57 (0.154 mmol) was dissolved in H_2O/*t*-BuOH (1:1) (1.0 mL) with vigorous stirring. The corresponding alkyne was added (1.0 eq), followed by sodium ascorbate (0.10 eq) as a freshly prepared solution (30 mg/mL in H_2O/*t*-BuOH 1:1) and $CuSO_4 \cdot 5H_2O$ (0.01 eq) also as a freshly prepared solution (30 mg/mL in H_2O). All reactions were carried out at r.t. and followed by TLC. Regular additions of freshly prepared solutions of sodium ascorbate and copper sulfate were done every 6 to 8 h. When no further changes were detected by TLC, alkyne (0.5 eq each time) was also added. This process was repeated according to TLC results until completion was achieved or no further changes were detected. The total amounts required for each alkyne are given in the individual descriptions of the compounds synthesized. Ice-cold water was added to the reaction mixture and the aqueous phase was extracted with EtOAc (3 x 3 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated. The product was purified by silica gel chromatography (PE/EA 2:1 gradient to 1:4, +1% Et_3N).

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-{2-[4-(1-hydroxy-1-methyl-ethyl)-[1,2,3]-triazol-1-yl]-ethyl}-3,4-*O*-isopropylidene-α-D-galactopyranoside (62), (CRDM020).

According to the general procedure, **57** (40 mg, 0.074 mmol) was reacted with 2-methyl-3-butin-2-ol (21.6 μL, 0.148 mmol) for 3 d to obtain **62** (37 mg, 81%) as white solid. $[\alpha]_D^{21}$ +53.9 (c = 0.97, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.05 (s, 9H, $C(CH_3)_3$), 1.32, 1.46 (2s, 6H, $C(CH_3)_2$), 1.63, 1.64 (s, 6H, $C[(CH_3)_2OH]$), 3.34 (s, 3H,

OCH_3), 3.40 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 7.8 Hz, 1H, H-2), 3.85 (dd, $J_{5,6}$ = 6.5 Hz, $J_{6a,6b}$ = 10.0 Hz, 1H, H-6a), 3.93 (dd, $J_{5,6}$ = 6.8 Hz, $J_{6a,6b}$ = 10.0 Hz, 1H, H-6b), 3.99-4.02 (m, 3H, H-5, H-2'), 4.22 (dd, $J_{2,3}$ = 7.7 Hz, $J_{3,4}$ = 5.5 Hz, 1H, H-3), 4.27 (dd, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 2.3 Hz, 1H, H-4), 4.54-4.56 (m, 2H, H-1'), 4.67 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 7.35-7.44, 7.68-7.72 (m, 11H, $2C_6H_5$, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.19 (**C**$(CH_3)_3$), 26.29 (C(**C**$H_3)_2$), 26.76 (3C, C(**C**$H_3)_3$), 28.39 (C(**C**$H_3)_2$), 30.37, 30.43 (C[(**C**$H_3)_2$OH]),50.17 (C-2'), 55.41 (OCH_3), 62.80 (C-6), 67.71 (C-5), 68.39 (**C**[$(CH_3)_2$OH]),69.39 (C-1'), 73.23 (C-4), 75.44 (C-3), 79.13 (C-2), 97.66 (C-1), 109.23 (**C**$(CH_3)_2$), 120.56, 127.64, 127.69, 129.72, 133.33, 133.43, 135.60, 155.36 (14C, $2C_6H_5$, C_2HN_3); Anal. calcd for $C_{33}H_{47}N_3O_7Si$ + 0.5 H_2O: C 62.44, H 7.62; Found: C 62.55, H 7.77.

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-{2-[4-(thiophen-3-yl)-[1,2,3]-triazol-1-yl]-ethyl}-3,4-*O*-isopropylidene-α-D-galactopyranoside (63), (CR VI 5). According to the general procedure, **57** (77.0 mg, 0.142 mmol) was reacted with 3-ethynyl thiophene (14.5 μL, 0.142 mmol) for 18 h to obtain **63** (89 mg, 97%) as white solid. $[\alpha]_D^{21}$ +55.0 (c = 0.66, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (s, 9H, $C(CH_3)_3$), 1.32, 1.42 (2s, 6H, $C(CH_3)_2$), 3.32 (s, 3H, OCH_3), 3.43 (m, 1H, H-2), 3.85 (m, 1H, H-6a), 3.93 (m, 1H, H-6b), 4.02 (m, 3H, H-5, H-2'), 4.25 (m, 2H, H-3, H-4), 4.60 (m, 2H, H-1'), 4.67 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 7.36-7.44, 7.69 (m, 13H, $2C_6H_5$, C_4H_3S), 8.01 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.19 (**C**$(CH_3)_3$), 26.29 (C(**C**$H_3)_2$), 26.75 (3C, C(**C**$H_3)_3$), 28.30 (C(**C**$H_3)_2$), 50.29 (C-2'), 55.39 (OCH_3), 62.81 (C-6), 67.73 (C-5), 69.31 (C-1'), 73.23, 75.34 (C-3, C-4), 79.21 (C-2), 97.50 (C-1), 109.26 (**C**$(CH_3)_2$), 114.51, 115.86, 120.76, 121.10, 125.84, 126.18, 127.63, 127.68, 129.71, 132.14, 133.33, 133.42, 135.59, 135.62, 143.83 (18C, $2C_6H_5$, C_4H_3S, C_2HN_3).

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-{2-[4-(3-amino-phenyl)-[1,2,3]-triazol-1-yl]-ethyl}-3,4-*O*-isopropylidene-α-D-galactopyranoside (64), (CR VI 4). According to the general procedure, **57** (78.2 mg, 0.144 mmol) was reacted with 3-ethynyl aniline (15.1 μL, 0.144 mmol) for 42 h to obtain **64** (94 mg, 98%) as white solid. $[\alpha]_D^{21}$ +84.3 (c = 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (s, 9H, $C(CH_3)_3$), 1.32, 1.42 (2s, 6H, $C(CH_3)_2$), 3.31 (s, 3H, OCH_3), 3.42 (dd, $J_{1,2}$ = 3.4 Hz,

$J_{2,3}$ = 7.5 Hz, 1H, H-2), (dd, $J_{5,6a}$ = 6.5 Hz, $J_{6a,6b}$ = 10.0 Hz, 1H, H-6a), 3.93 (dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 9.9 Hz, 1H, H-6b), 4.02 (m, 3H, H-5, H-2'), 4.26 (m, 2H, H-3, H-4), 4.60 (m, 2H, H-1'), 4.67 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 6.65, 7.14-7.20, 7.29, 7.35-7.44, 7.69 (m, 14H, $2C_6H_5$, C_6H_4), 8.06 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.20 (**C**$(CH_3)_3$), 26.30 (C(**C**$H_3)_2$), 26.76 (3C, C(**C**$H_3)_3$), 28.32 (C(**C**$H_3)_2$), 50.31 (C-2'), 55.42 (OCH_3), 62.83 (C-6), 67.73 (C-5), 69.32 (C-1'), 73.24, 75.36 (C-3, C-4), 79.22 (C-2), 97.52 (C-1), 109.27 (**C**$(CH_3)_2$), 112.19, 114.75, 116.04, 121.30, 127.64, 127.69, 129.67, 129.71, 131.82, 133.36, 133.45, 135.60, 135.63, 146.87, 147.72 (20C, $2C_6H_5$, C_5H_4, C_2HN_3).

Methyl 6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-*O*-{2-[4-(pyridin-3-yl)-[1,2,3]-triazol-1-yl]-ethyl}-3,4-*O*-isopropylidene-α-D-galactopyranoside (65), (CR VI 3).
According to the general procedure, **57** (83.6 mg, 0.154 mmol) was reacted with 3-ethynyl pyridine (32.5 mg, 0.309 mmol) for 28 h to obtain **65** (85 mg, 83%) as white solid. $[\alpha]_D^{21}$ +58.6 (c = 0.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (s, 9H, $C(CH_3)_3$), 1.32, 1.41 (2s, 6H, $C(CH_3)_2$), 3.32 (s, 3H, OCH_3), 3.43 (dd, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 7.6 Hz, 1H, H-2), 3.85 (dd, $J_{5,6a}$ = 6.5 Hz, $J_{6a,6b}$ = 10.0 Hz, 1H, H-6a), 3.93 (dd, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 10.0 Hz, 1H, H-6b), 4.04 (m, 3H, H-5, H-2'), 4.27 (m, 2H, H-3, H-4), 4.64 (m, 2H, H-1'), 4.69 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 7.35-7.43, 7.67-7.70, 8.22-8.24, 9.02 (m, 10H, C_6H_5, C_6H_4N, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.19 (**C**$(CH_3)_3$), 26.29 (C(**C**$H_3)_2$), 26.75 (3C, C(**C**$H_3)_3$), 28.34 (C(**C**$H_3)_2$), 50.44 (C-2'), 55.40 (OCH_3), 62.79 (C-6), 67.71 (C-5), 69.18 (C-1'), 73.24, 75.30 (C-3, C-4), 79.32 (C-2), 97.45 (C-1), 109.29 (**C**$(CH_3)_2$), 116.50 (CH-triazole), 121.71, 123.76, 127.07, 127.64, 127.69, 128.05, 129.72, 132.87, 133.32, 133.42, 135.59, 135.62, 144.54, 147.04, 149.08, (18C, $2C_6H_5$, C_5H_4N, C_2HN_3).

Methyl 2-*O*-{2-[4-(thiophen-3-yl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (68), (CR VI 7) and **Methyl 6-*O*-acetyl-2-*O*-{2-[4-(thiophen-3-yl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (69)**, (CR VI 7b).
63 (78.9 mg, 0.121 mmol), was stirred in 80% aqueous AcOH (5.2 mL) at 80°C. After cooling to r.t., the solvent was coevaporated five times with toluene. The crude material was dried at high vacuum and used without further purification. It was dissolved in THF (3.2 mL) and 1 M tetrabutylammonium fluoride solution in THF (0.24 mL, 0.24 mmol) was added. The mixture was stirred for 2 h. The solvent was

evaporated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 20:1 to 9:1), followed by P2 size exclusion chromatography (sephadex G-15 with H_2O as eluent) gave **68** (21mg, 47%) as white powder after a final lyophilization from H_2O/dioxane (1:1). The acetylated product **69** (27 mg, 53%) was also obtained as secondary product. **68**: $[\alpha]_D^{21}$ +82.3 (c = 0.38, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.89 (m, 1H, OH-6), 3.34 (s, 3H, OCH_3), 3.62 (s, 1H, OH-4), 3.65 (dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.8 Hz, 1H, H-2), 3.78 (t, $J_{5,6a}$ = $J_{5,6b}$ = 4.8 Hz,1H, H-5), 3.80-3.97 (m, 4H, H-3, H-6, OH-3), 4.00 (m, 1H, H-2'a), 4.09 (m, 2H, H-4, H-2'b), 4.52-4.62 (m, 2H, H-1'), 4.82 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 7.36-7.42, 7.66 (m, 3H, C_4H_3S), 7.90 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 50.35 (C-2'), 55.34 (OCH_3), 62.95 (C-6), 68.88, 68.94, 69.02 (C-3, C-5, C-1'), 70.54 (C-4), 78.05 (C-2), 97.64 (C-1), 120.87, 121.33, 125.74, 126.47, 131.54, 143.98 (6C, C_4H_3S, C_2HN_3); MS (ESI): Calcd for $C_{15}H_{22}N_3O_6S$ $[M+H]^+$: 372.12; Found m/z: 372.17; Anal. calcd for $C_{15}H_{21}N_3O_6S$ + 0.25 H_2O: C 47.93, H 5.72, N 11.17; Found: C 47.85, H 5.66, N 10.92. **69**: $[\alpha]_D^{21}$ +104.5 (c = 1.01, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.07 (s, 3H, Ac), 3.34 (s, 3H, OCH_3), 3.66 (dd, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 9.1 Hz, 1H, H-2), 3.91-4.01 (m, 4H, H-3, H-4, H-5, H-2'a), 3.80-3.97 (m, 4H, H-3, H-6, OH-3), 4.08 (m, 1H, H-2'b), 4.24 (dd, $J_{5,6a}$ = 7.3 Hz, $J_{6a,6b}$= 11.5 Hz, 1H, H-6a), 4.35 (dd, $J_{5,6b}$ = 5.3 Hz, $J_{6a,6b}$= 11.6 Hz, 1H, H-6b), 4.40-4.62 (m, 2H, H-1'), 4.80 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 7.36, 7.42, 7.65 (m, 3H, C_4H_3S), 7.89 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.87 (CH_3-Ac), 50.31 (C-2'), 55.29 (OCH_3), 63.35 (C-6), 67.63, 68.70, 68.96, 69.15 (C-3, C-4, C-5, C-1'), 78.00 (C-2), 97.49 (C-1), 120.82, 121.18, 125.77, 126.43, 131.64, 143.00 (C_4H_3S, C_2HN_3), 171.08 (CO); MS (ESI): Calcd for $C_{17}H_{24}N_3O_7S$ $[M+H]^+$: 414.13; Found m/z: 414.22; IR (NaCl) ν (cm^{-1}) 1739 s (CO); Anal. calcd for $C_{17}H_{23}N_3O_7S$ + 0.25 H_2O: C 49.39, H 5.61, N 10.16; Found: C 49.32, H 5.60, N 9.76.

Methyl 2-*O*-{2-[4-(1-hydroxy-1-methyl-ethyl)-[1,2,3]-triazol-1-yl]-ethyl-}α-D-galactopyranoside (66), (CR VI 1) and **Methyl 2-*O*-[2-(4-isopropenyl-[1,2,3]-triazol-1-yl)-ethyl]-α-D-galactopyranoside (67)**, (CR VI 2).

According to the procedure described for **68**, **62** (33 mg, 0.053 mmol) was stirred for 3 h in aqueous AcOH 80% (2.7 mL) and then reacted with 1 M tetrabutylammonium fluoride solution in THF (22.4 μL, 22.4 μmol) for 3 h. After work-up and purification, **66** (2.2 mg, 12%) was obtained as colorless oil, and **67** (4.4 mg, 39%) as by product

of **66** due to elimination under acidic conditions. **66**: $[\alpha]_D^{21}$ +35.4 (c = 0.22, $CHCl_3$); ^1H NMR (500 MHz, CD_3OD): δ 1.59 (s, 6H, $2CH_3$), 3.36 (s, 3H, OCH_3), 3.62 (dd, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.3 Hz, 1H, H-2), 3.67-3.74 (m, 2H, H-2, H-5, H-6), 3.80 (m, 1H, H-3), 3.88 (d, $J_{3,4}$ = 3.3 Hz, 1H, H-4), 4.03 (m, 2H, H-2'), 4.52-4.60 (m, 2H, H-1'), 4.80 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 7.99 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 31.07 (2C, $C[(CH_3)_2OH]$),51.96 (C-2'), 55.91 (OCH_3), 63.13 (C-6), 69.45 (C-1'), 70.49 (C-3), 71.97 (C-4), 72.55 (C-5), 79.19 (C-2), 99.64 (C-1), 119.36 (2C, C_2HN_3), 157.04 ($C[(CH_3)_2OH]$);MS (ESI): Calcd for $C_{14}H_{25}N_3NaO_7$ $[M+Na]^+$: 370.16; Found m/z: 370.27; Anal. calcd for $C_{14}H_{25}N_3O_7$ + 0.25 H_2O: C 47.79, H 7.30, N 11.94; Found: C 47.53, H 7.17 N 11.81. **67**: $[\alpha]_D^{21}$ +55.1 (c = 0.34, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 2.13 (s, 3H, CH_3), 3.36 (s, 3H, OCH_3), 3.66 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 3.79 (t, $J_{5,6a}$ = $J_{5,6b}$ = 4.9 Hz, 1H, H-5), 3.85-3.96 (m, 3H, H-3, H-6), 3.98-4.02 (m, 1H, H-2'a), 4.06-4.10 (m, 1H, H-2'b), 4.13 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.50-4.61 (m, 2H, H-1'), 4.82 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.10 (s, 1H, H-1"a), 5.68 (s, 1H, H-1"b), 7.74 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.62 (CCH_3), 50.22 (C-2'), 55.32 (OCH_3), 62.85 (C-6), 68.82, 69.07 (C3, C-5), 69.11 (C-1'), 70.41 (C-4), 78.19 (C-2), 97.72 (C-1), 112.61 (CH_2CCH_3), 120.94, 133.47 (C_2HN_3), 149.00 (CH_2CCH_3); MS (ESI): Calcd for $C_{14}H_{23}N_3NaO_6$ $[M+Na]^+$: 352.15; Found m/z: 352.23.

Methyl 2-*O*-{2-[4-(3-amino-phenyl)-[1,2,3]-triazol-1-yl]-ethyl-}-α-D-galactopyranoside (70), (CR VI 9).

According to the procedure described for 68, 64 (64 mg, 0.097 mmol) was stirred for 9 h in aqueous AcOH 80% (5 mL) and then reacted with 1 M tetrabutylammonium fluoride solution in THF (0.20 mL, 0.20 mmol) overnight. After work-up and purification, 70 (9 mg, 24%) was obtained as yellowish oil. $[\alpha]_D^{21}$ +95.2 (c = 0.14, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 3.30 (s, 3H, OCH_3), 3.63-3.73 (m, 4H, H-2, H-5, H-6), 3.82 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 3.88 (d, $J_{3,4}$ = 3.3 Hz, 1H, H-4), 4.03-4.10 (m, 2H, H-2'), 4.58-4.66 (m, 2H, H-1'), 4.77 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 6.71, 7.11-7.19 (m, 4H, C_6H_4), 8.38 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 52.11 (C-2'), 55.88 (OCH_3), 63.12 (C-6), 70.44 (C-1'), 70.92 (C-3), 71.55 (C-4), 72.53 (C-5), 79.14 (C-2), 99.50 (C-1), 113.76, 116.85, 116.90, 123.59, 131.07 (8C, C_6H_4, C_2HN_3); Anal. calcd for $C_{17}H_{24}N_4O_6$ + 1.25 H_2O: C 50.68, H 6.63, N 13.90; Found: C 50.63, H 6.34 N 13.90.

Methyl 2-*O*-{2-[4-(pyridin-3-yl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (71), (CR VI 8).

According to the procedure described for **68**, **65** (78 mg, 0.12 mmol) was stirred overnight in aqueous AcOH 80% (5.5 mL) and then reacted with 1 M tetrabutylammonium fluoride solution in THF (0.24 mL, 0.24 mmol) for 2 h. After work-up and purification, **71** (27 mg, 60%) was obtained as yellow solid. $[\alpha]_D^{21}$ +177.4 (c = 0.12, $CHCl_3$); ^1H NMR (500 MHz, CD_3OD): δ 3.30 (s, 3H, OCH_3), 3.63-3.74 (m, 4H, H-2, H-5, H-6), 3.83 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 3.89 (d, $J_{3,4}$ = 2.6 Hz, 1H, H-4), 4.05-4.12 (m, 2H, H-2'), 4.62-4.72 (m, 2H, H-1'), 4.80 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 7.52, 8.28, 8.51, 8.64, 9.01 (m, 5H, C_5H_4N, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 52.56 (C-2'), 56.16 (OCH_3), 63.45 (C-6), 70.54 (C-1'), 71.20 (C-3), 71.89 (C-4), 72.88 (C-5), 79.43 (C-2), 99.79 (C-1), 124.94, 126.37, 129.60, 135.60, 145.85, 147.95, 150.23 (C_5H_4N, C_2HN_3); MS (ESI): Calcd for $C_{16}H_{23}N_4O_6$ $[M+H]^+$: 367.16; Found m/z: 367.09; Anal. calcd for $C_{16}H_{22}N_4O_6$ + 0.5 H_2O: C 51.20, H 6.18; Found: C 51.12, H 6.34.

General procedure for the synthesis of 1,4-disubstituted [1,2,3]-triazoles from 58.

Compound **58** (36.6 mg, 0.139 mmol) was dissolved in H_2O/*t*-BuOH (1:1) (1.8 mL) with vigorous stirring. The corresponding alkyne was added (1.0 eq), followed by sodium ascorbate (0.10 eq) as freshly prepared solution (30 mg/mL in H_2O/*t*-BuOH 1:1) and $CuSO_4 \cdot 5H_2O$ (0.01 eq) also as freshly prepared solution (20 mg/mL in H_2O). All reactions were carried out at r.t. and followed by TLC. Regular additions of freshly prepared solutions of sodium ascorbate and copper sulfate were done every 6 to 8 h. When no more changes were detected by TLC, alkyne (0.5 eq each time) was also added. This process was repeated according to TLC results until completion was achieved or no further changes were detected. The total amounts required for each alkyne are given in the individual descriptions of the compounds synthesized. The reaction mixture was concentrated under reduced pressure and the crude material was purified by silica gel chromatography (DCM/MeOH 20:1 or 9:1), followed by P2 size exclusion chromatography (sephadex G-15 with H_2O as eluent) or filtration through Gelman Acrodiscs (0.2 μm).

Methyl 2-*O*-{2-[4-(1-hydroxy-1-methyl-ethyl)-1,2,3-triazol-1-yl]-ethyl-}α-D-galactopyranoside (66), (CR VI 1)**.**

According to the general procedure, **58** (30.5 mg, 0.114 mmol) was reacted with 2-methyl-3-butin-2-ol (16.5 μL, 0.171 mmol) for 48 h. After work-up and purification it was lyophilized from H_2O to obtain **66** (23 mg, 57%) as white solid. Analytical data coincident with data presented above for **66** in the synthesis of 1,4-disubstituted 1,2,3-triazoles from **57**.

Methyl 2-*O*-[2-(4-methylaminomethyl-[1,2,3]-triazol-1-yl)-ethyl]-α-D-galactopyranoside (72), (CRVI 10).

According to the general procedure, **58** (40.7 mg, 0.155 mmol) was reacted with *N*-methyl propargylamine (13.5 μL, 0.155 mmol) for 42 h. After work-up it was purified by RP chromatography (5% gradient MeOH in H_2O) followed by P2 size exclusion chromatography and a final lyophilization from H_2O to obtain **72** (27 mg, 51%) as pale green solid. $[\alpha]_D^{21}$ +82.7 (c = 1.32, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 2.72 (s, 3H, $CH_2NHC\mathbf{H_3}$), 3.28 (s, 3H, OCH_3), 3.57 (m, 1H, H-2), 3.69 (m, 2H, H-5, H-6a), 3.72-3.80 (m, 2H, H-3, H-6b), 3.91 (s, 1H, H-4), 4.10 (m, 2H, H-2'), 4.37 (s, 2H, $C\mathbf{H_2}NHCH_3$), 4.64 (m, 2H, H-1'), 4.86 (d, $J_{1,2}$ = 2.6 Hz, 1H, H-1), 8.22 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, D_2O): δ 32.33 ($CH_2NH\mathbf{C}H_3$), 42.90 ($\mathbf{C}H_2NHCH_3$), 50.96 (C-2'), 55.05 (OCH_3), 61.49 (C-6), 68.99 (C-1'), 69.05, 69.52, 70.93 (C-3, C-4, C-5), 77.14 (C-2), 97.45 (C-1), 127.21, 138.24 (C_2HN_3); MS (ESI): Calcd for $C_{13}H_{25}N_4O_6$ $[M+H]^+$: 333.18; Found m/z: 333.09.

Methyl 2-*O*-{2-[4-(4-tolyl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (73), (CR VI 12).

According to the general procedure, **58** (35.1 mg, 0.133 mmol) was reacted with 4-ethynyltoluene (87.5 μL, 0.669 mmol) for 88 h. After work-up and purification **58** (13 mg, 37%) was recovered and **73** (18 mg, 57%) was obtained as white solid. $[\alpha]_D^{21}$ +92.9 (c = 0.90, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (s, 3H, CH_3), 3.35 (s, 3H, OCH_3), 3.69 (m, 1H, H-2), 3.79 (m, 1H, H-5), 3.86 (m, 1H, H-6a), 3.95 (m, 2H, H-3, H-6b), 4.03 (m, 1H, H-2'a), 4.12 (m, 2H, H-4, H-2'b), 4.23-4.65 (m, 2H, H-1'), 4.85 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 7.22, 7.70 (m, 4H, C_6H_4), 7.92 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.30 (CH_3), 50.34 (C-2'), 55.38 (OCH_3), 63.25 (C-6), 68.79, 68.93 (C-3, C-5), 69.02 (C-1'), 70.76 (C-4), 78.13 (C-2), 97.64 (C-1), 125.63, 127.60,

129.58, 138.18 (8C, C_6H_4, C_2HN_3); MS (ESI): Calcd for $C_{18}H_{26}N_3O_6$ $[M+H]^+$: 380.18; Found m/z: 380.29; Anal. calcd for $C_{18}H_{25}N_3O_6$: C 56.99, H 6.64; Found: C 57.20, H 6.72.

Methyl 2-*O*-{2-[4-(3-chloro-phenyl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (74), (CR VI 11).

According to the general procedure, **58** (36.0 mg, 0.137 mmol) was reacted with 3-chloro-1-ethynyl benzene (87.5 μL, 0.689 mmol) for 89 h. After work-up and purification it was lyophilizated from dioxane to obtain **74** (38 mg, 74%) as white powder. $[\alpha]_D^{21}$ +95.6 (c = 0.32, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.39 (s, 3H, OCH_3), 3.70 (dd, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 9.9 Hz, 1H, H-2), 3.79 (m, 1H, H-5), 3.86 (dd, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 11.8 Hz, 1H, H-6a), 3.92-3.98 (m, 2H, H-3, H-6b), 4.03 (m, 1H, H-2'a), 4.11 (m, 2H, H-4, H-2'b), 4.56-4.65 (m, 2H, H-1'), 4.89 (d, $J_{1,2}$ = 3.1 Hz, 1H, H-1), 7.32, 7.69, 7.80 (m, 4H, C_6H_4), 8.03 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 50.47 (C-2'), 55.36 (OCH_3), 63.20 (C-6), 68.88, 68.94, 69.05 (C-3, C-5, C-1'), 70.76 (C-4), 78.06 (C-2), 97.61 (C-1), 121.45, 123.75, 125.74, 128.24, 130.22, 132.20, 134.85 (8C, C_6H_4, C_2HN_3); MS (ESI): Calcd for $C_{17}H_{23}ClN_3O_6$ $[M+H]^+$: 400.13; Found m/z: 400.21; Anal. calcd for $C_{17}H_{22}ClN_3O_6$: C 51.07, H 5.55, N 10.51; Found: C 51.15, H 5.61, N 9.95.

Methyl 2-*O*-{2-[4-(benzotriazol-1-ylmethyl)-[1,2,3]-triazol-1-yl]-ethyl}-α-D-galactopyranoside (75), (CRVI 6).

According to the general procedure, **58** (36.6 mg, 0.139 mmol) was reacted with 1-propargyl-1*H*-benzotriazole (22.5 mg, 0.139 mmol) for 21 h. After work-up and purification it was lyophilized from H_2O/dioxane (1:1) to obtain **75** (51 mg, 87%) as white solid. $[\alpha]_D^{21}$ +45.9 (c = 0.59, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.28 (s, 3H, OCH_3), 3.58 (dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.8 Hz, 1H, H-2), 3.74 (t, $J_{5,6a}$ = $J_{5,6b}$ = 4.8 Hz, 1H, H-5), 3.82-3.90 (m, 4H, H-3, H-6, H-2'a), 3.96 (m, 1H, H-2'b), 4.07 (d, $J_{3,4}$ = 2.2 Hz, 1H, H-4), 4.46 (m, 1H, H-1'a), 4.54 (m, 1H, H-1'b), 4.74 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 7.35-7.48, 7.33, 7.93, 7.97 (m, 5H, $C_6H_4N_3$, C_2HN_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 43.63 (C**C**H_2N), 50.51 (C-2'), 55.24 (OCH_3), 62.78 (C-6), 68.71, 68.91, 69.05 (C-3, C-5, C-1'), 70.59 (C-4), 77.80 (C-2), 97.59 (C-1), 110.13, 119.73, 124.42, 124.62, 127.89, 132.76, 141.81 (8C, $C_6H_4N_3$, C_2HN_3); MS (ESI): Calcd for

$C_{18}H_{24}N_6NaO_6$ $[M+Na]^+$: 443.17; Found m/z: 443.15; Anal. calcd for $C_{18}H_{24}N_6O_6$ + 0.25 H_2O: C 50.88, H 5.75; Found: C 51.06, H 5.85.

Synthesis of scaffold 93 for solid phase synthesis

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucose (77), (CR III 2), **(α:β 1:2).**
To a stirred mixture of D-glucosamine hydrochloride (100 g, 464 mmol), water (1.2 L) and sodium bicarbonate (78.0 g, 929 mmol) was added a solution of phthalic anhydride (68.0 g, 459 mmol) in dioxane (900 mL). After 4 days the reaction was acidified to pH 4-5 with 2 N HCl (90 mL). The solvents were evaporated under reduced pressure.
The crude material (75.7 g, 168 mmol) was suspended in acetic anhydride (600 mL) and sodium acetate (55.0 g, 670 mmol) was added. The mixture was refluxed at 140°C for 20 min. After cooling to r.t., the reaction was diluted with water (600 mL) and neutralized with $NaHCO_3$, followed by extraction with DCM (4 x 1 L). The combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the residue by MPLC (PE/EA 3:2) gave **77** (55.4 g, 57%) as white foam. $[\alpha]_D^{21}$ +71.4 (*c* = 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.58, 1.87, 2.00, 2.04, 2.05, 2.06, 2.09, 2.13 (8s, 12H of **77α**, 4Ac**;** 12H of **77β**, 4Ac), 4.03 (m, 1H of **77β**, H-5), 4.13 (m, 2H of **77α**, H-5, H-6a; 1H of **77β**, H-6a), 4.37 (m, 1H of **77α**, H-6b; 1H of **77β**, H-6b), 4.47 (dd, $J_{1,2}$ = 8.9 Hz, $J_{2,3}$ = 10.6 Hz, 1H of **77β**, H-2), 4.72 (dd, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 11.6 Hz, 1H of **77α**, H-2), 5.15-5.24 (m, 1H of **77α**, H-4; 1H of **77β**, H-4), 5.89 (m, 1H of **77β**, H-3), 6.29 (d, $J_{1,2}$ = 3.4 Hz, 1H of **77α**, H-1) 6.51-6.59 (m, 1H of **77α**, H-3; 1H of **77β**, H-1), 7.75, 7.86 (m, 4H of **77α**, C_6H_4; 4H of **77β**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.41, 20.62, 20.66, 20.75, 20.78, 21.00 (8C, 4Ac, **α/β**), 52.81 (C-2, **α**), 53.50 (C-2, **β**), 61.53 (C-6, **α/β**), 67.00 (C-3, **α**), 68.29 (C-4, **β**), 69.38 (C-4, **α**), 70.18 (C-5, **α**), 70.51 (C-3, **β**), 72.54 (C-5, **β**), 89.77 (C-1, **β**), 90.53 (C-1, **α**), 123.75, 123.82, 131.22, 134.49, 134.51 (12C, C_6H_4, **α/β**), 167.38, 168.65, 169.33, 169.49, 169.55, 169.79, 170.03, 170.67 (12C, CO, **α/β**); Missing signals are due to overlapping or low resolution.

3,4,6-Tri-*O*-acetyl-1-bromo-2-deoxy-2-phthalimido-D-glucose (78), (CR III 4), **(α:β 1:4).**

Compound **77** (10.2 g, 21.4 mmol) was dissolved under argon in DCM (150 mL). At 0°C a solution of 33% HBr in AcOH (30 mL, 0.17 mol) was added dropwise during 30 min. The reaction was stirred at r.t. for 5 h with light exclusion. The reaction was quenched at 0°C with ice-cold saturated aqueous $NaHCO_3$. The mixture was extracted with DCM (4 x 200 mL) and the combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure. **78** (10.8 g, quantitative) was obtained as the α/β-mixture (1:4) and used in the next step without further purification. $[\alpha]_D^{21}$ +70.4 (*c* = 0.99, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.87, 1.90, 2.04. 2.07, 2.12, 2.14 (6s, 9H of **78α**, 3Ac; 9H of **78β**, 3Ac), 3.97 (m, 1H of **78β**, H-5), 4.19 (m, 1H of **78α**, H-5; 1H of **78β**, H-6a), 4.34 (dd, $J_{5,6b}$ = 4.7 Hz, $J_{6a,6b}$ = 12.5 Hz, 1H of **78β**, H-6b), 4.44 (dd, $J_{5,6a}$ = $J_{5,6b}$ = 3.4 Hz, $J_{6a,6b}$ = 14.7 Hz, 2H of **78α**, H-6), 4.61-4.70 (m, 1H of **78α**, H-2; 1H of **78β**, H-2), 5.16 (t, $J_{3,4}$ = 9.4 Hz, 1H of **78α**, H-4), 5.27 (m, 1H of **78β**, H-4), 5.77 (m, 1H of **78β**, H-3), 6.41 (d, $J_{1,2}$ = 9.6 Hz, 1H of **78β**, H-1), 6.57 (d, $J_{1,2}$ = 3.7 Hz, 1H of **78α**, H-1), 6.66 (m, 1H of **78α**, H-3), 7.77, 7.88 (m, 8H of **78α**, C_6H_4; 8H of **78β**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.39, 20.59, 20.70, 20.78 (6C, 3Ac, α/β), 56.27 (C-2, α), 58.14 (C-2, β), 61.71 (C-6, α/β), 67.60 (C-3, α), 68.13 (C-4, β), 68.86 (C-4, α), 70.61 (C-3, β), 72.45 (C-5, α), 76.89 (C-5, β), 77.29 (C-1, β), 87.19 (C-1, α), 123.91, 134.60 (12C, C_6H_4, α/β), 169.32, 169.98, 170.68 (10C, 5CO, α/β); Missing signals due to overlapping or low resolution.

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (79), (CRIII 5).

A mixture of $CaSO_4$ (8.2 g, 60 mmol) and Ag_2CO_3 (6.7 g, 24 mmol) in MeOH (250 mL) was stirred with light exclusion for 30 min. A solution of compound **78** (10.1 g, 20 mmol) in MeOH (40 mL) was added. The mixture was stirred at r.t. for 23 h and then filtered over a pad of celite. The solution was concentrated under reduced pressure and the residue was redissolved in DCM (100 mL). The solution was extracted with 2% aqueous NH_4OH (2 x 50 mL) and water (50 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1) afforded **79** (7.90 g, 87%). $[\alpha]_D^{21}$ +44.9 (*c* = 0.99, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ 1.86, 2.03, 2.12 (3s, 9H, 3Ac), 3.45 (s, 3H,

OCH_3), 3.87 (m, 1H, H-5), 4.30 (d, $J_{5,6a}$ = $J_{6a,6b}$ = 8.9 Hz, 1H, H-6a), 4.34 (m, 2H, H-2, H-6b), 5.18 (t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.30 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 5.78 (t, $J_{2,3}$ = $J_{3,4}$ = 10.1 Hz, 1H, H-3), 7.74, 7.85 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 18.34, 18.53, 18.67 (3C, 3Ac), 52.39 (C-2), 54.97 (OCH_3), 59.85 (C-6), 66.84 (C-3), 68.67 (C-4), 69.73 (C-5), 96.93 (C-1), 121.51, 132.17 (6C, C_6H_4), 167.38, 168.08 (5C, CO); MS (ESI): Calcd for $C_{21}H_{23}NNaO_{10}$ [M+Na]$^+$: 472.12; Found m/z: 472.15.

Methyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (80) (CRIII 6).

Compound **79** (8.87 g, 19.7 mmol) was suspended in MeOH (200 mL) and NaOMe was added until pH 9. The reaction was stirred overnight at r.t. After neutralization with amberlyste 15 ion-exchange resin, the reaction was filtered over a pad of celite and the solvent was evaporated. **80** (7.10 g, quantitative) was obtained as yellowish oil and used in the next step without further purification. $[\alpha]_D^{21}$ +5.2 (*c* = 1.08, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.38 (s, 3H, OCH_3), 3.44 (m, 1H, H-5), 3.68 (t, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, 1H, H-4), 3.88 (m, 2H, H-6), 4.06 (m, 1H, H-2), 4.27 (t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.12 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 7.69, 7.80 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 56.78, 56.91 (C-2, OCH_3), 61.39 (C-6), 71.02 (C-3), 71.39 (C-4), 75.74 (C-5), 99.36 (C-1), 123.45, 131.73, 134.05 (6C, C_6H_4), 168.62 (2C, CO); MS (ESI): Calcd for $C_{15}H_{17}NNaO_7$ [M+Na]$^+$: 346.09; Found m/z: 346.00.

Methyl 2-deoxy-2-phthalimido-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (81), (CR III 7).

A solution of **80** (3.42 g, 11.0 mmol) in pyridine (67 mL) was cooled to -38°C. A catalytic amount of DMAP was added to the solution. Pivaloyl chloride (3.4 mL, 28 mmol) was added during 30 min with vigorous stirring under argon. After 17 h the reaction was warmed to 0°C and stirred for additional 2 h. The solvent was evaporated and the residue dissolved in DCM (60 mL). The solution was extracted with 0.1 N HCl (2 x 20 mL) and saturated aqueous $NaHCO_3$ (30 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 4:1, +1% EtOH) gave **81** (3.62 g, 67%). $[\alpha]_D^{21}$ -7.2 (*c* = 1.06, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00, 1.26 (2s, 18H, 2C$(CH_3)_3$), 3.05 (s,1H, OH-4), 3.43 (s, 3H, OCH_3), 3.59 (t, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, 1H, H-

4), 3.75 (m, 1H, H-5), 4.23 (t, $J_{1,2}$ = $J_{2,3}$= 10.0 Hz, 1H, H-2), 4.45 (m, 2H, H-6), 5.20 (d, $J_{1,2}$ = 9.4 Hz, 1H, H-1), 5.59 (t, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, 1H, H-3), 7.73, 7.79 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 26.83, 27.02, 27.23 (6C, 2C(**C**H_3)$_3$), 38.77, 38.98 (2**C**(CH_3)$_3$), 54.25 (C-2), 56.82 (OCH_3), 63.22 (C-6), 70.59 (C-4), 73.32 (C-3), 74.38 (C-5), 98.98 (C-1), 123.52, 131.47, 134.26 (6C, C_6H_4), 178.98, 179.26 (2CO); MS (ESI): Calcd for $C_{25}H_{33}NNaO_9$ $[M+Na]^+$: 514.21; Found m/z: 514.23.

Methyl 2-deoxy-2-phthalimido-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (82), (CRIII 8).

A solution of **81** (2.73 g, 5.56 mmol) in DCE/pyridine (2:1, 49 mL) was cooled to 0°C. A catalytic amount of DMAP was added under argon, followed by dropwise addition of trifluoromethan sulfonic anhydride (1.5 mL, 8.3 mmol) during 40 min. The reaction was stirred for 17 h, then water (5.5 mL) was added. The mixture was warmed to 80°C and stirred under argon for additional 5 h. After cooling to r.t., the reaction was diluted with DCM (40 mL) and extracted with 5% HCl (2 x 40 mL), saturated aqueous $KHCO_3$ (2 x 40 mL) and water (40 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 5:1 to 3:1, +1% EtOH) gave **82** (1.61 g, 59%) as yellowish oil. $[\alpha]_D^{21}$ -9.7 (*c* = 0.92, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21, 1.32 (2s, 18H, 2C(CH_3)$_3$), 3.45 (s, 3H, OCH_3), 4.07 (m, 1H, H-5), 4.16 (m, 1H, H-6a), 4.24 (m, 1H, H-6b), 4.34 (m, 1H, H-2), 4.63 (m, 1H, H-3), 5.18 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 5.34 (m, 1H, H-4), 7.73, 7.84 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 27.49, 27.59 (6C, 2C(**C**H_3)$_3$), 39.15, 39.78 (2**C**(CH_3)$_3$), 54.34 (C-2), 57.23 (OCH_3), 62.11 (C-6), 68.57 (C-3), 69.71 (C-4), 71.53 (C-5), 99.94 (C-1), 132.17, 134.49 (6C, C_6H_4), 178.33, 179.33 (2CO); MS (ESI): Calcd for $C_{25}H_{33}NNaO_9$ $[M+Na]^+$: 514.21; Found m/z: 514.27.

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (83a), (CRIII 10).

Galactoside **82** (1.88 g, 3.82 mmol) was dissolved in MeOH (78 mL). A solution of NaOMe in MeOH (0.5 M, 6.8 mL) was added and the reaction was stirred overnight at r.t. After neutralization with amberlyste 15 ion-exchange resin and filtration over a pad of celite, the solvent was evaporated under reduced pressure. The residue (1.27 g, 3.72 mmol) was suspended in acetic anhydride (80 mL) and sodium acetate (1.22

g, 14.9 mmol) was added. The mixture was refluxed at 140°C for 15 min. After cooling to r.t., the reaction was diluted with water (40 mL), neutralized with solid $NaHCO_3$ and extracted with DCM (4 x 40 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1) gave **83a** (1.67 g, quantitative). $[\alpha]_D^{21}$ +0.37 (*c* = 0.74, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD): δ 1.85, 2.07, 2.20 (3s, 9H, 3Ac), 3.46 (s, 3H, OCH_3), 4.09 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, 1H, H-5), 4.19 (dd, $J_{5,6a}$ = 6.8 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, H-6a), 4.25 (dd, $J_{5,6b}$ = 6.6 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, H-6b), 4.53 (m, 1H, H-2), 5.24 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 5.48 (m, 1H, H-4), 5.79 (m, 1H, H-3), 7.74, 7.80 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 20.93, 21.09 (3C, 3Ac), 51.71 (OCH_3), 57.42 (C-2), 61.81 (C-6), 67.16 (C-4), 68.48 (C-3), 71.17 (C-5), 99.78 (C-1), 131.90, 134.68 (6C, C_6H_4), 170.26, 170.86 (5C, CO); MS (ESI): Calcd for $C_{21}H_{23}NNaO_{10}$ $[M+Na]^+$: 472.12; Found m/z: 472.13.

Methyl 2-deoxy-2-phthalimido-β-D-galactopyranoside (83), (CRIII 11).

83a (5.81 g, 12.9 mmol) was suspended in MeOH (230 mL) and NaOMe was added until pH 9. The reaction was stirred overnight at r.t. After neutralization with amberlyste 15 ion exchange resin, the reaction was filtered over celite and the solvent was evaporated. **83** (4.34 g, quantitative) was obtained as white foam and used in the next step without further purification. $[\alpha]_D^{21}$ +1.6 (*c* = 0.99, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.38 (s, 3H, OCH_3), 3.61 (m, 1H, H-5), 3.74-3.81 (m, 2H, H-6), 3.92 (m, 1H, H-4), 4.31 (m, 1H, H-2), 4.38 (m, 1H, H-3), 5.00 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1), 7.77-7.82 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 55.42 (C-2), 56.93 (OCH_3), 62.51 (C-6), 69.55 (C-3), 70.01 (C-4), 77.06 (C-5), 101.17 (C-1), 123.98, 124.26, 133.09, 135.45 (6C, C_6H_4), 169.80, 170.16 (2CO); MS (ESI): Calcd for $C_{15}H_{17}NNaO_7$ $[M+Na]^+$: 346.09; Found m/z: 346.02.

Methyl 2-deoxy-2-phthalimido-6-*O*-(toluene-4-sulfonyl)-β-D-galactopyranoside (84), (CR III 33).

Compound **83** (40 mg, 0.12 mmol) was dissolved in DCM (0.3 mL). Dibutyltin oxide (0.6 mg, 2.4 μmol) was added followed by tosyl chloride (24 mg, 0.12 mmol) and Et_3N (17 μL, 0.12 mmol). The reaction was stirred at r.t. for 4 h, then filtered and concentrated *in vacuo*. Purification by silica gel chromatography (PE/EA 1:1, DCM/MeOH 20:1) gave **84** (25.5 mg, 43%) as white solid, **84b** (8 mg, 11%) (3,6-*O*-

tosylated), and recovered **83** (11 mg, 28%). The product **84** was obtained in 60% yield considering the starting material recovered. **84**: ^{1}H NMR (500 MHz, CD_3OD): δ 2.46 (s, 3H, CH_3-Ts), 2.79 (d, $J_{3,OH}$ = 8.8 Hz, 1H, OH-3), 2.85 (d, $J_{4,OH}$ = 5.2 Hz, 1H, OH-4), 3.37 (s, 3H, OCH_3), 3.91 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.2 Hz, 1H, H-5), 4.04 (m, 1H, H-4), 4.19-4.26 (m, 2H, H-2, H-6a), 4.32 (m, 1H, H-6b), 4.39 (m, 1H, H-3), 5.06 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 7.37, 7.71, 7.82 (m, 8H, $2C_6H_4$); ^{13}C NMR (125 MHz, CD_3OD): δ 22.08 (CH_3-Ts), 54.54 (C-2), 57.18 (OCH_3), 68.11 (C-6), 68.51 (C-4), 68.75 (C-3), 72.44 (C-5), 99.82 (C-1), 123.87, 128.41, 130.44, 132.12, 132.83, 134.55, 145.70 (12C, $2C_6H_4$), 168.99 (2CO).

Methyl 2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-β-D-galactopyranoside (85), (CR III 34).

Galactoside **88** (7.20 g, 12.0 mmol) was dissolved in THF (245 mL) and 1 M tetrabutylammonium fluoride solution in THF (24 mL, 24 mmol) was added. The mixture was stirred at r.t. for 3 h. The solvent was evaporated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:3, +0.5% Et_3N) gave **85** (3.70 g, 85%) as a colorless oil. $[\alpha]_D^{21}$ +33.6 (*c* = 1.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.63 (2s, 6H, $C(CH_3)_2$), 3.43 (s, 3H, OCH_3), 3.91 (m, 1H, H-6a), 4.06 (m, 2H, H-5, H-6b), 4.25 (m, 2H, H-2, H-4), 4.80 (m, 1H, H-3), 5.00 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 7.71, 7.83 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.75, 28.15 ($C(\mathbf{C}H_3)_2$), 55.25 (C-2), 56.95 (OCH_3), 60.64 (C-6), 73.70, 74.14, 74.51 (C-3, C-4, C-5), 99.37 (C-1), 110.98 ($\mathbf{C}(CH_3)_2$), 132.13, 134.31 (6C, C_6H_4); Missing signals are due to low resolution. MS (ESI): Calcd for $C_{18}H_{21}NNaO_7$ $[M+Na]^+$: 386.12; Found m/z: 386.03; Anal. calcd for $C_{18}H_{21}NO_7$ + 0.75 H_2O: C 57.37; H 6.02; N 3.72; Found : C 57.64; H 6.05; N 3.47.

Methyl 2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-D-galactopyranoside (86), (CRIII 29A).

Compound **83** (40 mg, 0.12 mmol) was dissolved under argon in dry CH_3CN (2.6 mL). 2,2-Dimethoxypropane (23 μL, 3.8 mmol) was added with vigorous stirring followed by a catalytic amount of camphor sulfonic acid in acetonitrile (100 μL, 10.2 mg/mL, 0.0440 mmol). After stirring at r.t. for 55 h the reaction was neutralized by addition of Et_3N (2 mL). The material was concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA 1:1, +1% Et_3N) to afford **86**

(43 mg, 96%) as colorless oil. ^{1}H NMR (500 MHz, CD_3OD): δ 1.49, 1.52 (2s, 6H, $C(CH_3)_2$), 2.44 (m, 1H, OH-3), 3.44 (s, 3H, OCH_3), 3.52 (m, 1H, H-5), 4.03 (m, 1H, H-6a), 4.14 (m, 1H, H-6b), 4.23 (d, $J_{3,4}$ = 1.8 Hz, 1H, H-4), 4.38 (m, 2H, H-2, H-3), 5.12 (m, 1H, H-1), 7.72, 7.83 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, CD_3OD): δ 19.27, 29.62 (2C, $C(\mathbf{C}H_3)_2$), 54.98 (C-2), 57.11 (OCH_3), 63.00 (C-6), 67.04 (C-5), 68.15, 68.29 (C-3, C-4), 99.46 (C-1), 99.76 ($\mathbf{C}(CH_3)_2$), 123.49, 123.96, 132.39, 134.37, 168.83 (8C, C_6H_4, 2CO).

Methyl 2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-2-phthalimido-β-D-galactopyranoside (87), (CR III 31).

83 (4.9 g, 15 mmol) was dissolved in DCM (58 mL) under argon. To this solution were added *tert*-butyl-diphenylchlorosilane (4.7 mL, 18.2 mmol), Et_3N (2.6 mL, 18 mmol) and DMAP (74 mg, 0.61 mmol). The reaction was stirred for 12 h at r.t. and then concentrated under reduced pressure. The residue was redissolved in DCM (50 mL) and washed with water (2 x 25 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 2:1 to 4:3) gave **87** (8.1 g, 96%). $[\alpha]_D^{21}$ +7.9 (*c* = 0.74, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (s, 9H, $C(CH_3)_3$), 2.68 (d, $J_{3,OH}$ = 9.6 Hz, 1H, OH-3), 3.14 (d, $J_{4,OH}$ = 4.9 Hz, 1H, OH-4), 3.42 (s, 3H, OCH_3), 3.70 (m, 1H, H-5), 3.99 (m, 2H, H-6), 4.19 (m, 1H, H-4), 4.35 (m, 2H, H-2, H-3), 5.09 (d, $J_{1,2}$ = 6.9 Hz, 1H, H-1), 7.39-7.48, 7.70-7.75, 7.82-7.84 (m, 14H, $2C_6H_5$, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.28 ($\mathbf{C}(CH_3)_3$), 26.90 (3C, $C(\mathbf{C}H_3)_3$), 54.80 (C-2), 56.62 (OCH_3), 63.83 (C-6), 69.13 (C-3), 69.58 (C-4), 73.94 (C-5), 99.60 (C-1), 127.98, 128.02, 130.11, 132.02, 132.72, 132.94, 134.12, 135.69, 135.81 (18C, $2C_6H_5$, C_6H_4); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{31}H_{35}NNaO_7Si$ $[M+Na]^+$: 584.21; Found m/z: 584.23; Anal. calcd for $C_{31}H_{35}NO_7Si$ + H_2O: C 64.23; H 6.43; N 2.42; Found : C 64.43; H 6.40; N 2.31.

Methyl 2-deoxy-6-*O*-[(1,1-dimethylethyl)diphenylsilyl]-3,4-*O*-isopropylidene-2-phthalimido-β-D-galactopyranoside (88), (CR III 32).

Compound **87** (87 mg, 0.15 mmol) was dissolved under argon in dry CH_3CN (3 mL). 2,2-Dimethoxypropane (28 μL, 0.23 mmol) was added with vigorous stirring followed by a catalytic amount of *p*-toluene sulfonic acid monohydrate in acetonitrile (100 μL, 8.80 mg/mL, 4.60 μmol). After stirring for 16 h at r.t. the reaction was neutralized by

addition of Et_3N (1 mL). The solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA 4:1, +1% Et_3N) to afford **88** (90 mg, 96%) as white foam. $[\alpha]_D^{21}$ +13.5 (*c* = 0.71, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.07 (s, 9H, $C(CH_3)_3$), 1.33, 1.64 (2s, 6H, $C(CH_3)_2$), 3.39 (s, 3H, OCH_3), 3.98-4.14 (m, 3H, H-5, H-6), 4.27 (m, 1H, H-2), 4.37 (m, 1H, H-4), 4.80 (m, 1H, H-3), 4.98 (d, $J_{1,2}$ = 4.3 Hz, 1H, H-1), 7.38-7.45, 7.70-7.74, 7.83 (m, 14H, $2C_6H_5$, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.64 (**C**$(CH_3)_3$), 26.85 (C(**C**$H_3)_2$), 27.14 (3C, C(**C**$H_3)_3$), 28.40 (C(**C**$H_3)_2$), 55.62 (C-2), 56.88 (OCH_3), 63.09 (C-6), 73.54 (C-4), 73.99 (C-3), 74.51 (C-5), 99.49 (C-1), 110.71 (**C**$(CH_3)_2$), 128.07, 128.14, 130.14, 132.38, 133.72, 133.81, 134.40, 135.99, 136.04 (18C, $2C_6H_5$, C_6H_4), 171.54 (2CO); MS (ESI): Calcd for $C_{34}H_{39}NNaO_7Si$ $[M+Na]^+$: 624.24; Found m/z: 624.25; Anal. calcd for $C_{34}H_{39}NO_7Si$: C 67.86; H 6.53; N 2.33; Found : C 67.73; H 6.54; N 2.32.

Methyl 2-deoxy-3,4-*O*-isopropylidene-6-*O*-methanesulfonyl-2-phthalimido-β-D-galactopyranoside (89), (CR III 35).

Compound **85** (3.38 g, 9.29 mmol) was dissolved in pyridine (90 mL). Mesyl chloride (1.45 mL, 18.6 mmol) was added dropwise at 0°C. After 15 min the reaction was warmed to r.t. and stirring continued for 2.5 h. The reaction was diluted with EtOAc (50 mL) and extracted with 1 N HCl (2 x 50 mL), saturated aqueous $NaHCO_3$ solution (2 x 50 mL) and brine (40 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated. Silica gel chromatography (PE/EA 1:1, +1% Et_3N) of the residue gave **89** (3.90 g, 95%) as white solid. $[\alpha]_D^{21}$ +25.0 (*c* = 1.24, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.63 (2s, 6H, $C(CH_3)_2$), 3.09 (s, 3H, SCH_3), 3.42 (s, 3H, OCH_3), 4.22-4.30 (m, 3H, H-2, H-4, H-5), 4.82 (m, 1H, H-3), 5.00 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 7.72, 7.84 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.89, 28.25 (C(**C**$H_3)_2$), 37.82 (SCH_3), 55.11 (C-2), 57.26 (OCH_3), 69.20 (C-6), 71.46 (C-5), 73.29 (C-4), 74.47 (C-3), 99.44 (C-1), 111.35 (**C**$(CH_3)_2$), 132.23, 134.53 (6C, C_6H_4); Missing signals are due to low resolution. MS (ESI): Calcd for $C_{19}H_{23}NNaO_9S$ $[M+Na]^+$: 464.10; Found m/z: 464.11;

Methyl 6-azido-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-β-D-galactopyranoside (90), (CR III 36).

89 (1.21 g, 2.74 mmol) was dissolved under argon in dry DMF (130 mL). Sodium azide (890 mg, 13.7 mmol) was added followed by 15-crown-5 (217 μL, 1.10 mmol). The reaction was vigorously stirred under argon at 100°C for 5 d. The reaction mixture was then diluted at 0°C with EtOAc (70 mL) and washed with ice-cold water (2 x 70 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic phases were dried with Na_2SO_4, filtered, concentrated and the resulting syrup was purified by silica gel chromatography (PE/EA 8:1 to 3:1, +1% Et_3N) to afford **90** (1.02 g, 96%) as white solid. $[\alpha]_D^{21}$ +14.9 (*c* = 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32, 1.64 (2s, 6H, $C(CH_3)_2$), 3.39-3.44 (m, 4H, H-6a, OCH_3), 4.12 (m, 1H, H-5), 4.17 (m, 1H, H-4), 4.27 (t, $J_{1,2}$ = $J_{2,3}$ = 8.9 Hz, 1H, H-2), 4.80 (m, 1H, H-3), 5.01 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 7.72, 7.84 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.87, 28.28 ($C(\mathbf{C}H_3)_2$), 51.58 (C-6), 55.28 (C-2), 57.16 (OCH_3), 73.39, 74.08, 74.48 (C-3, C-4, C-5), 99.47 (C-1), 111.23 ($\mathbf{C}(CH_3)_2$), 132.31, 134.48 (6C, C_6H_4); Missing signals are due to low resolution. MS (ESI): Calcd for $C_{18}H_{20}N_4NaO_6$ $[M+Na]^+$: 411.13; Found m/z: 411.11; IR (NaCl) ν 2100 s ($-N=N^+-N^-$), 1610 vs (CO) (cm^{-1}); Anal. calcd for $C_{18}H_{20}N_4O_6$: C 55.67; H 5.19; N 14.43; Found : C 55.67; H 5.16; N 14.23.

Methyl 6-azido-2-deoxy-2-phthalimido-β-D-galactopyranoside (91), **(**CR III 37).

Compound **90** (1.24 g, 3.19 mmol) was stirred at 80°C in 80% aqueous AcOH (100 mL) for 2 h. After cooling to r.t., the solvents were coevaporated with toluene. Purification by silica gel chromatography (DCM/MeOH 9:1) gave **91** (1.10 g, quantitative) as white foam. $[\alpha]_D^{21}$ -37.2 (*c* = 0.52, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.33 (dd, $J_{5,6a}$ = 3.5 Hz, $J_{6a,6b}$ = 12.5 Hz, 1H, H-6a), 3.45 (m, 4H, OCH_3, OH-3), 3.62 (d, $J_{4,OH}$ = 5.4 Hz, OH-4), 3.75-3.83 (m, 2H, H-5, H-6b), 3.97 (m, 1H, H-4), 4.26 (m, 1H, H-2), 4.40 (m, 1H, H-3), 5.10 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 7.70, 7.79 (m, 4H, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.64 (C-6), 54.57 (C-2), 57.28 (OCH_3), 69.06 (C-3), 69.81 (C-4), 74.85 (C-5), 99.89 (C-1), 123.87, 132.09, 134.55 (6C, C_6H_4) 169.15 (2CO); MS (ESI): Calcd for $C_{15}H_{16}N_4NaO_6$ $[M+Na]^+$: 371.10; Found m/z: 371.04; IR (NaCl) ν 2097 s ($-N=N^+-N^-$),1705 vs (CO) (cm^{-1}); Anal. calcd for $C_{15}H_{16}N_4O_6$: C 51.72; H 4.63; N 16.09; Found : C 51.84; H 4.86; N 15.62.

Methyl 3,4-*O*-[(1*RS*)-4-(Allyl carboxylate)-benzylidene]-6-azido-2-deoxy-2-phthalimido-β-D-galactopyranoside (92a/b), (CR III 38a/b), **(a:b 7:2).**

A soxhlet extractor with activated powdered molecular sieves (3 Å), was conditioned under argon at 105°C with dry CH_3CN (100 mL) for 20 h. After cooling to r.t., dry **91** (417 mg, 1.20 mmol) dissolved in CH_3CN (5 mL) and **2** (240 mg, 1.02 mmol) dissolved in CH_3CN (5 mL) were added. Toluene-4-sulfonic acid monohydrate (0.96 mL of a 10 mg/mL solution in CH_3CN) was added to the mixture. The mixture was refluxed at 105°C for 66 h. After cooling to r.t., the reaction was neutralized by addition of Et_3N (30 mL) (pH 7) and the mixture was concentrated under reduced pressure. Purification by silica gel chromatography (PE/EA 8:1 to 4:1, +1% Et_3N) afforded **92a/b** (339 mg, 64%) as isomeric mixture. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.39-3.49 (m, 4H of **92a**, H-6a, OCH_3; 4H of **92b**, H-6a, OCH_3), 3.83-3.88 (m, 1H of **92a**, H-6b; 1H of **92b**, H-6b), 4.10 (m, 1H of **92b**, H-5), 4.17 (m, 1H of **92b**, H-4), 4.21 (m, 1H of **92a**, H-5), 4.28-4.33 (m, 2H of **92a**, H-2, H-4), 4.36 (t, $J_{1,2} = J_{2,3} = 8.8$ Hz, 1H of **92b**, H-2), 4.83 (m, 2H of **92a**, $CH_2CHC\mathbf{H_2}O$; 2H of **92b**, $CH_2CHC\mathbf{H_2}O$), 5.01-5.13 (m, 2H of **92a**, H-1, H-3; 2H of **92b**, H-1, H-3), 5.29 (m, 1H of **92a**, Ha of $C\mathbf{H_2}CHCH_2O$; 1H of **92b**, Ha of $C\mathbf{H_2}CHCH_2O$), 5.41 (m, 1H of **92a**, Hb of $C\mathbf{H_2}CHCH_2O$; 1H of **92b**, Hb of $C\mathbf{H_2}CHCH_2O$), 5.96 (s, 1H of **92a**, CHO_2), 6.05 (m, 1H of **92a**, $CH_2C\mathbf{H}CH_2O$; 1H of **92b**, $CH_2C\mathbf{H}CH_2O$), 6.35 (s, 1H of **92b**, CHO_2), 7.48, 7.68 (m, 2H of **92a**, 2H of C_6H_4; 2H of **92b**, 2H of C_6H_4), 7.74, 7.86 (m, 4H of **92a**, Phth; 4H of **92b**, Phth), 8.05, 8.15 (m, 2H of **92a**, 2H of C_6H_4; 2H of **92b**, 2H of C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.46 (C-6, **92a**), 51.80 (C-6, **92b**), 52.53 (C-2, **92b**), 55.97 (C-2, **92a**), 57.18, 57.28 (OCH_3, **92a/b**), 66.05 ($CH_2CH\mathbf{C}H_2O$, **92a/b**), 73.18 (C-5, **92a**), 73.57 (C-5, **92b**), 74.37 (C-4, **92a/b**), 76.08, 76.51 (C-3, **92a/b**), 99.37, 99.55 (C-1, **92a/b**), 103.14 (CHO_2, **92b**), 105.01 (CHO_2, **92a**), 118.73 ($\mathbf{C}H_2CHCH_2O$, **92a/b**), 123.93, 126.6, 127.49, 130.20, 130.30, 131.70, 132.21, 132.50, 132.58, 134.58, 134.67, 141.32 (30C, $CH_2\mathbf{C}HCH_2O$, **92a/b**; C_6H_4, **92a/b**; Phth, **92a/b**), 166.27 (CO, **92a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{26}H_{24}N_4NaO_8$ [M+Na]$^+$: 543.15; Found m/z: 543.19; IR (NaCl) ν 2102 s (-N=N$^+$-N$^-$),1716 vs (CO) (cm^{-1}); Anal. calcd for $C_{26}H_{24}N_4O_8$: C 60.00; H 4.65; N 10.76; Found : C 59.57; H 4.72; N 10.37.

Methyl 6-azido-2-deoxy-2-phthalimido-3,4-*O*-[(1*RS*)-4-(triethylammonium carboxylate)-benzylidene]-β-D-galactopyranoside (93a/b), (CR III 39a/b), **(a:b 7:2).**

Compound **92a/b** (571 mg, 1.10 mmol) was dissolved in degassed THF. *N*-Methyl morpholine (1.2 mL, 11 mmol) was added to the solution followed by $Pd(PPh_3)_4$ (127 mg, 0.110 mmol). The reaction was excluded from light and then pyrrolidine (0.92 mL, 11 mmol) was added at 0°C. The reaction was stirred for 30 min at 0°C and then concentrated under reduced pressure. Silica gel chromatography (DCM/MeOH 20:1, +1% Et_3N) of the crude material **93a/b** (634 mg, 86%) as white foam. $[\alpha]_D^{21}$ +91.0 (*c* = 0.99, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 1.12 (m, 9H of **93a**, CH_3 of Et_3NH^+; 9H of **93b**, CH_3 of Et_3NH^+),2.79 (m, 6H of **93a**, CH_2 of Et_3NH^+; 6H of **93b**, CH_2 of Et_3NH^+), 3.38-3.44 (m, 4H of **93a**, H-6a, OCH_3; 4H of **93b**, H-6a, OCH_3), 3.83 (m, 1H of **93a**, H-6b; 1H of **93b**, H-6b), 4.14 (m, 1H of **93b**, H-5), 4.17 (m, 1H of **93a**, H-5; 1H of **93b**, H-4), 4.21 (dd, $J_{3,4}$ = 5.8 Hz, $J_{4,5}$ = 2.4 Hz, 1H of **93a**, H-4), 4.35 (m, 1H of **93a**, H-2; 1H of **93b**, H-2), 4.95-5.04 (m, 2H of **93a**, H-1, H-3; 2H of **93b**, H-1, H-3), 5.86 (s, 1H of **93a**, CHO_2), 6.30 (s, 1H of **93b**, CHO_2), 7.35, 7.55 (m, 2H of **93a**, 2H of C_6H_4; 2H of **93b**, 2H of C_6H_4), 7.7.67, 7.80 (m, 4H of **93a**, Phth; 4H of **93b**, Phth), 8.01, 8.10 (m, 2H of **93a**, 2H of C_6H_4; 2H of **93b**, 2H of C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.30 ($(\mathbf{C}H_3CH_2)_3NH^+$, **93a/b**), 45.80 ($(CH_3\mathbf{C}H_2)_3NH^+$, **93a/b**), 51.45 (C-6, **93a**), 51.87 (C-6, **93b**), 52.55 (C-2, **93b**), 56.05 (C-2, **93a**), 57.09, 57.18 (OCH_3, **93a/b**), 73.35, 73.76 (C-5, **93a/b**), 74.09, 74.18 (C-3, **93a/b**), 75.89 (C-4, **93b**), 76.35 (C-4, **93a**), 99.35 (C-1, **93b**), 99.35 (C-1- **93a**), 103.82 (CHO_2, **93b**), 105.75 (CHO_2, **93a**), 123.85, 125.91, 126.97, 129.91, 130.00, 132.19, 134.45, 134.58, 137.94, 138.90, (28C, C_6H_4, **93a/b**; Phth, **93a/b**), 172.65 (CO, **93a/b**); Missing signals are due to overlapping or low resolution. MS (ESI): Calcd for $C_{23}H_{20}N_4NaO_8$ $[M+Na]^+$: 503.12; Found m/z: 503.21; IR (NaCl) ν 2103 s ($-N=N^+-N^-$), 1716 vs (CO) (cm^{-1}).

Test compounds for solid phase synthesis

Methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (12), (CR II 1).

Methyl β-D-galactopyranoside (400 mg, 2.06 mmol) was dissolved under argon in DMF (7.5 mL). 2,2-Dimethoxypropane (380 μL, 3.10 mmol) was added with vigorous stirring followed by a catalytic amount of *p*-toluene sulfonic acid monohydrate (17 mg, 0.090 mmol). After stirring at 80°C for 4 h the reaction was neutralized by addition of

Et_3N (3 mL). The solution was concentrated under reduced pressure. Purification by silica gel chromatography (DCM/MeOH 20:1) afforded **12** (481 mg, quantitative). $[\alpha]_D^{21}$ -16.0 (*c* = 0.80, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35, 1.52 (2s, 6H, $C(CH_3)_2$), 3.55 (m, 4H, H-2, OCH_3), 3.86 (m, 2H, H-5, H-6a), 4.01 (m, 1H, H-6b), 4.11 (m, 2H, H-1, H-3), 4.17 (d, $J_{3,4}$ = 5.3 Hz, 1H, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.73, 28.49 ($C(\mathbf{C}H_3)_2$), 57.53 (OCH_3), 62.89 (C-6), 73.83, 74.14, 74.41 (C-2, C-4, C-5), 79.25 (C-3), 103.68 (C-1).

Methyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (94), (CR II 2).

NaH (509 mg of 55% NaH in oil, 11.6 mmol) was washed with hexane (3 x 10 mL) under argon and dried in high vacuum. After suspending the NaH in dry DMF (5 mL), benzyl bromide (1.2 mL, 10 mmol) was added dropwise over 30 min at 0°C. Then a solution of **12** (929 mg, 3.96 mmol) in DMF (7 mL) was added dropwise at 0°C. The reaction was warmed to r.t. and stirred for 4 h. The reaction was quenched by addition of thiocarbamide (303 mg, 3.97 mmol), and stirred for 30 min. After dilution with EtOAc (10 mL), the organic phase was washed with 1 M KH_2PO_4 (10 mL) and brine (10 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA 5:1 to 4:1) to obtain **94** (1.03 g, 63%) which was immediately used in the following step. Analytical data were in accordance with the literature.

Methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside (95), (CR II 3).

94 (815 mg, 1.97 mmol), was stirred in 80% aqueous AcOH (20 mL) at 80°C for 3 h. After cooling to r.t., the solvents were coevaporated five times with toluene. Purification by silica gel chromatography (PE/EA 1:1) gave **95** (695 mg, 94%). Analytical data were in accordance with the literature.

Methyl 3,4-*O*-[(1*RS*)-4-(Allyl carboxylate)-benzylidene]-2,6-di-*O*-benzyl-β-D-galactopyranoside (96a/b), (CR II 4a/b), **(a:b 1:1).**

Compound **95** (323 mg, 0.863 mmol) was dissolved in DCM (3 mL) and a catalytic amount of *p*-toluenesulfonic acid (12 mg, 0.063 mmol) was added. Compound **1** was dissolved in DCM (3 mL) and added to the previous solution. Additional DCM (4mL) was added. The reaction was stirred at r.t. for 24 h. The solvent was evaporated

under reduced pressure and the residue purified by silica gel chromatography (PE/EA 3:1) to obtain **96a/b** (197 mg, 63%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.37 (m, 1H of **96a**, H-2; 1H of **96b**, H-2), 3.56, 3.60 (2s, 3H of **96a**, OCH_3; 3H of **96b**, OCH_3), 3.84 (m, 1H of **96a**, H-6a; 1H of **96b**, H-6a), 3.90 (m, 1H of **96a**, H-6b; 1H of **96b**, H-6b), 4.02 (m, 1H of **96a**, H-5; 1H of **96b**, H-5), 4.17-4.36 (m, 3H of **96a**, H-1, H-3, H-4; 3H of **96b,** H-1, H-3, H-4), 4.57-4.75 (m, 4H of **96a**, $2CH_2$ of Bn; 4H of **96b**, $2CH_2$ of Bn), 4.84 (m, 2H of **96a**, CH_2CHCH_2O; 2H of **96b**, CH_2CHCH_2O), 5.31 (m, 1H of **96a**, Ha of CH_2CHCH_2O; 1H of **96b**, Ha of CH_2CHCH_2O), 5.43 (m, 1H of **96a**, Hb of CH_2CHCH_2O; 1H of **96b**, Hb of CH_2CHCH_2O), 5.93, 5.96 (2s, 1H of **96a**, CHO_2; 1H of **96b**, CHO_2), 6.02-6.09 (m, 1H of **96a**, CH_2CHCH_2O; 1H of **96b**, CH_2CHCH_2O), 7.24-7.41 (m, 10H of **96a**, $2C_6H_5$; 10H of **96b**, $2C_6H_5$), 7.45, 8.01, 8.09, 8.10 (m, 4H of **96a**, C_6H_4; 4H of **96b**, C_6H_4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 57.00, 57.04 (OCH_3, **96a/b**), 65.75 (CH_2CHCH_2O, **96a/b**), 69.35 (C-6, **96a/b**), 71.98,72.39 (C-5, **96a/b**), 73.55, 73.82 ($4CH_2$ of Bn, **96a/b**), 76.37, 78.84 (C-3, **96a/b**; C-4, **96a/b**), 79.88, 80.14 (C-2, **96a/b**), 103.82, 104.00 (4C, C-1, **96a/b**; CHO_2, **96a/b**), 118.39 (CH_2CHCH_2O, **96a/b**), 126.42, 126.77, 126.84, 126.91, 127.75, 127.84, 127.90, 127.94, 128.17, 128.34, 128.38, 128.57, 129.82, 129.88, 130.92, 132.31, 138.15, 142.35 (36C, $2C_6H_5$, **96a/b**; C_6H_4, **96a/b)**, 166.05 (CO, **96a/b**).

Solid phase synthesis of methyl β-D-galactose-derivatives

General procedure for lantern loading

Lantern swelling: The lanterns were immersed for 30 min in DMF.

Lantern activation: The lanterns were treated with 20% Et_3N in DMF (3 x 10 min).

Standard washing procedure: The lanterns were washed with DMF (3 x 10 min) and DCM (3 x 10 min).

Loading: The coupling solution consisted of **93a/b** [3.3 eq x 35 μmol x (no. of lanters + 0.5)] and HOBt [3.3 eq x 35 μmol x (no. of lanters + 0.5)] in DMF [0.5 mL x (no. of lanters + 0.5)]. Of the coupling solution, 0.5 mL per well was pipetted in a 96-well plate followed by DIC (17.7 μL, 3.3 eq x 35 μmol). After 2 min of pre-activation, the lantern was added and the plate was left at r.t. in the shaker for 15 to 21 h. The lanterns were then washed according to the above washing procedure.

General procedure for cleavage of the phthalimide

The loaded lanterns were treated with hydrazine monohydrate (0.15 mL/lantern, 80 eq) in dry MeOH (2 mL/lantern) in a sealed tube (bombenrohr) under argon at 80°C (3 x 4 h and 2 x overnight). The lanterns were then washed with MeOH (3x 10 min) followed by the standard washing procedure.

General procedure for amino acid coupling

Two stock solutions were freshly prepared. Solution **A** consisted of the Fmoc protected amino acid [4 eq x 35 μmol x (no. of lanterns + 1)] and HOBt [4 eq x 35 μmol x (no. of lanterns + 1)] in DMF [250 μL x (no. of lanterns +1)]. Solution **B** consisted of HBTU [3.6 eq x 35 μmol x (no. of lanterns + 1)] in DMF [250 μL x (no. of lanterns + 1)]. 250 μL of freshly prepared stock solution **A**, 250 μL of freshly prepared stock solution **B,** and 48 μL of DIPEA (8 eq x 35 μmol) were combined in a 96-well plate. After 2 min of pre-activation the lantern was added. The coupling procedure was repeated five times, each time fresh solutions **A** + **B** were used. The first coupling was left on the shaker overnight, followed by three couplings of 4 h each. Finally, the fifth coupling was run overnight. The lanterns were then washed according to the standard washing procedure.

General procedure for the synthesis of 1,4-disubstituted 1,2,3-triazoles

Stock solutions of sodium ascorbate (113 mg/mL) and $CuSO_4 \cdot 5H_2O$ (28.5 mg/mL) were freshly prepared. Per well (of a 96-well plate) a 2.4 M solution of the corresponding alkyne (30 eq x 35 μmol) in H_2O/*t*-BuOH (440 μL) was added, followed by sodium ascorbate stock solution (20 μL) and $CuSO_4 \cdot 5H_2O$ stock solution (10 μL). The lantern was added and the plate was left at r.t. on the shaker. Five additions of freshly prepared stock solutions (equal amounts as the initial one) were performed during one day every 3 h. The whole process was repeated on four consecutive days. The last two additions performed at the last two days, included a catalytic amount of CuCl. The lanterns were then washed with DMF (5 x 10 min) and DCM (5 x 10 min).

General procedure for the cleavage of Fmoc The lanterns were treated with 20% piperidine in DMF (3 x 10 min). The standard washing procedure was then applied.

General procedure for the cleavage from the solid phase

Each lantern was treated with 80% aqueous AcOH containing 2% TFA (2 mL) (2 x 15 h). The solvents were evaporated under reduced pressure.

General procedure for purification

Products were purified by chromatography on RP-18 (5% gradient MeOH in H_2O). After concentration under reduced pressure and filtration, the samples were lyophilized from H_2O/dioxane (1:1).

Methyl 2-(amino-acetamido)-6-azido-2-deoxy-β-D-galactopyranoside (98), (CR III 57).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-glycine and cleaved from the solid phase after Fmoc cleavage. During the evaporation of the solvents, partial decomposition occurred (drastic color change of the solution) and after purification **98** (2 mg, 21%) was obtained as pale green powder. ^{1}H NMR (500 MHz, CD_3OD): δ 3.24 (m, 1H, H-6a), 3.47 (s, 3H, OCH_3), 3.62 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.69 (m, 4H, H-5, H-6b, CH_2 of Gly), 3.77 (d, $J_{3,4}$ = 3.1 Hz, 1H, H-4), 3.97 (m, 1H, H-2), 4.32 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ 41.85 (CH_2 of Gly), 53.07 (C-6), 54.17 (C-2), 57.14 (OCH_3), 70.14 (C-4), 73.43 (C-3), 76.40 (C-5), 103.96 (C-1), MS (ESI): Calcd for $C_9H_{17}N_5NaO_5$ $[M+Na]^+$: 298.11; Found m/z: 298.03; IR (NaCl) ν 2105 s ($-N=N^+-N^-$);1680 vs (CO) (cm^{-1}). Melting point: 115°C (decomposition).

Methyl 2-[(*S*)-2-amino-3-methyl-butyrylamido]-6-azido-2-deoxy-β-D-galactopyranoside (100), (CR III 54).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-valine and cleaved from solid phase after Fmoc cleavage. Compound **100** (14 mg, 100% recovery, ≥98% purity) was obtained as the acetate. After purification and a final lyophilization from dioxane/H_2O (1:1) **100** (7 mg, 67%) was obtained as white powder. $[\alpha]_D^{21}$ -4.9 (*c* = 0.52, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.09 (m, 6H, $2CH_3$ of Val), 2.20 (m, 1H, C**H**$(CH_3)_2$), 3.24 (m, 1H, H-6a), 3.48 (s, 3H, OCH_3), 3.62-3.70 (m, 4H, H-3, H-5, H-6b, $CHNH_2$), 3.75 (d, $J_{3,4}$ = 3.2 Hz, 1H, H-4), 3.90 (m, 1H, H-2), 4.42 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ 17.84, 19.02 (CH(**C**$H_3)_2$), 31.41 (**C**$H(CH_3)_2$), 52.47 (C-6), 54.08 (C-2),

56.86 (OCH_3), 59.49 ($CHNH_2$), 69.61 (C-4), 72.63 (C-3), 75.80 (C-5), 102.94 (C-1); MS (ESI): Calcd for $C_{12}H_{24}N_5O_5$ $[M+H]^+$: 318.18; Found m/z: 318.16; Melting point: 121°C-125°C.

Methyl 2-(amino-acetamido)-6-{4-butyl-[1,2,3]-triazol-1-yl}-2-deoxy-β-D-galactopyranoside (102), (CR III 48).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-glycine, reacted with 1-hexine and cleaved from solid phase after Fmoc cleavage. Each day one extra aliquot of 1-hexine was added (30 eq, 135 μL) due to its fast evaporation. The acetate salt of **102** (15.1 mg, 100% recovery) was obtained as the only product. After purification and a final lyophilization from dioxane/H_2O (1:1) **102** (7 mg, 56%) was obtained as white powder. $[\alpha]_D^{21}$ +15.5 (*c* = 0.49, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.93 (t, $J_{3',4'}$ = 7.3 Hz, 3H, CH_3 of $CH_2CH_2CH_2CH_3$), 1.31-1.40 (m, 2H, H-3'), 1.60-1.66 (m, 2H, H-2'), 2.69 (t, $J_{1',2'}$ = 7.3 Hz, 2H, H-1'), 3.28 (s, 3H, OCH_3), 3.62-3.68 (m, 3H, H-3, CH_2-Gly), 3.84 (d, $J_{3,4}$ = 2.8 Hz, 1H, H-4), 3.92 (m, 1H, H-5), 3.99 (m, 1H, H-2), 4.19 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.65 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 2H, H-6), 7.75 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 14.08 (C-4'), 22.82 (C-3'), 25.53 (C-1'), 32.49 (C-2'), 41.07 (CH_2 of Gly), 51.71 (C-6), 53.61 (C-2), 56.88 (OCH_3), 69.01 (C-4), 72.83 (C-3), 74.87 (C-5), 102.95 (C-1), 124.31 (2C, C_2HN_3); MS (ESI): Calcd for $C_{15}H_{27}N_5NaO_5$ $[M+Na]^+$: 380.19; Found m/z: 380.20.

Methyl 2-(amino-acetamido)-2-deoxy-6-{4-(napht-2-yl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (103), (CR III 50).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-glycine, reacted with 1-ethynyl naphtalene and cleaved from the solid phase after Fmoc-cleavage. Compound **103** (17 mg, 100% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **103** (7 mg, 47%) was obtained as white powder. $[\alpha]_D^{21}$ +19.4 (*c* = 0.50, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 3.33 (s, 3H, OCH_3), 3.71 (m, 3H, H-3, CH_2 of Gly), 3.94 (d, $J_{3,4}$ = 3.0 Hz, 1H, H-4), 4.03-4.10 (m, 2H, H-2, H-5), 4.28 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.84 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 2H, H-6), 7.49-7.57, 7.68, 7.94, 8.22 (m, 8H, C_2HN_3, $C_{10}H_7$); ^{13}C NMR (125 MHz, CD_3OD): δ

41.08 (CH_2 of Gly), 51.79 (C-6), 53.55 (C-2), 56.71 (OCH_3), 69.87 (C-4), 72.85 (C-3), 74.78 (C-5), 103.21 (C-1), 125.99, 126.37, 127.14, 128.29, 129.31 (12C, C_2HN_3, $C_{10}H_7$); MS (ESI): Calcd for $C_{21}H_{26}N_5O_5$ $[M+H]^+$: 428.19; Found m/z: 428.24; Melting point: 142°C-146°C.

Methyl 2-[(*S*)-2-amino-propionamido]-6-{4-butyl-[1,2,3]-triazol-1-yl}-2-deoxy-β-D-galactopyranoside (104), (CR III 47).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 1-hexine and cleaved from the solid phase after Fmoc-cleavage. Each day one extra aliquot of 1-hexine was added (30 eq, 135 μL) due to its fast evaporation. Compound **104** (12.4 mg, 82% recovery) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **104** (11.5 mg, 89%) was obtained as white powder. $[\alpha]_D^{21}$ +28.3 (*c* = 0.72, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.93 (t, $J_{3',4'}$ = 7.3 Hz, 3H, H-4'), 1.33-1.40 (m, 2H, H-3'), 1.53 (d, 3H, CH_3 of Ala), 1.61-1.67 (m, 2H, H-2'), 2.70 (t, $J_{1',2'}$ = 7.6 Hz, 2H, H-1'), 3.28 (s, 3H, OCH_3), 3.67 (m, 1H, H-3), 3.84 (d, $J_{3,4}$ = 2.4 Hz, 1H, H-4), 3.89-3.98 (m, 3H, H-2, H-5, CH of Ala), 4.22 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.65 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 2H, H-6), 7.76 (s,1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 14.48 (C-4'), 18.17 (CH_3 of Ala), 23.55 (C-3'), 26.28 (C-1'), 33.20 (C-2'), 50.91 (CH of Ala), 52.44 (C-6), 54.42 (C-2), 57.20 (OCH_3), 70.33 (C-4), 73.15 (C-3), 75.28 (C-5), 103.89 (C-1), 124.76 (2C, C_2HN_3), 172.09 (CO); MS (ESI): Calcd for $C_{16}H_{29}N_5NaO_5$ $[M+Na]^+$: 394.21; Found m/z: 394.31; Melting point: 123°C-125°C.

Methyl 2-[(*S*)-2-amino-propionamido]-2-deoxy-6-{4-(napht-2-yl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (105), (CR III 49).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 1-ethynyl naphtalene and cleaved from the solid phase after Fmoc-cleavage. Compound **105** (16.4 mg, 93% recovery, ≥98% purity) was obtainedas the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **105** (9 mg, 58%) was obtained as white powder. $[\alpha]_D^{21}$ +22.9 (*c* = 0.44, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.53 (d, 3H, CH_3 of Ala), 3.33 (s, 3H, OCH_3), 3.71 (dd, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.91 (m, 2H, H-4, CH of Ala), 4.01 (m, 1H, H-2), 4.09 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 1H, H-5),

4.30 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.84 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 2H, H-6), 7.49-7.57, 7.68, 7.94 (m, 7H, $C_{10}H_7$), 8.33 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 18.03 (CH_3 of Ala), 49.91 (CH of Ala), 52.09 (C-6), 53.72 (C-2), 56.79 (OCH_3), 69.47 (C-4), 72.91 (C-3), 75.09 (C-5), 102.97 (C-1), 126.20, 127.33, 128.49, 129.35 (12C, C_2HN_3, $C_{10}H_7$); MS (ESI): Calcd for $C_{22}H_{27}N_5NaO_5$ $[M+Na]^+$: 464.19; Found m/z: 464.26; Melting point: 145°C-148°C.

Methyl 2-[(*S*)-2-amino-propionamido]-6-{4-(3-chloro-phenyl)-[1,2,3]-triazol-1-yl}-2-deoxy-β-D-galactopyranoside (106), (CR III 46).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with3-chloro-1-ethynyl benzene and cleaved from the solid phase after Fmoc-cleavage. Compound **106** (13.6 mg, 80% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **106** (8 mg, 53%) was obtained as white powder. $[\alpha]_D^{21}$ +14.5 (*c* = 0.40, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.52 (d, 3H, CH_3 of Ala), 3.28 (s, 3H, OCH_3), 3.68 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.0 Hz, 1H, H-3), 3.89 (m, 2H, H-4, CH of Ala), 3.99 (m, 2H, H-2, H-5), 4.25 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.75 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 2H, H-6), 7.35, 7.42, 7.72 (m, 4H, C_6H_4), 8.41 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 18.16 (CH_3 of Ala), 50.90 (CH of Ala), 52.71 (C-6), 54.41 (C-2), 57.19 (OCH_3), 70.31 (C-4), 73.19 (C-3), 75.18 (C-5), 103.89 (C-1), 124.47, 125.27, 126.82, 129.62, 132.04, 134.16, 136.40, 147.61 (C_2HN_3, C_6H_4); MS (ESI): Calcd for $C_{18}H_{25}ClN_5O_5$ $[M+Na]^+$: 426.15; Found m/z: 426.18; Melting point: 139°C-141°C.

Methyl 2-[(*S*)-2-amino-propionamido]-6-{4-(benzotriazol-1-ylmethyl)-[1,2,3]-triazol-1-yl}-2-deoxy-β-D-galactopyranoside (107), (CR III 42).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 1-propargyl-1*H*-benzotriazole and cleaved from the solid phase after Fmoc-cleavage. Compound **107** (10.6 mg, 60% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **107** (7 mg, 46%) was obtained as white powder. $[\alpha]_D^{21}$ +32.4 (*c* = 0.37, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.48 (d, 3H, CH_3 of Ala), 2.90 (s, 3H, OCH_3), 3.59 (dd, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 3.82-

3.90 (m, 4H, H-2, H-4, H-5, CH of Ala), 4.04 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.67 (m, 2H, H-6), 6.06 (d, $J_{Ha,Hb}$ = 6.4 Hz, 2H, CCH_2N), 7.43, 7.55, 7.85, 7.96 (m, 4H, $C_6H_4N_3$), 8.07 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 18.00 (CH_3 of Ala), 44.20 ($C\mathbf{C}H_2N$), 49.83 (CH of Ala), 52.32 (C-6), 53.96 (C-2), 56.65 (OCH_3), 69.88 (C-4), 73.73 (C-3), 74.71 (C-5), 103.32 (C-1), 111.63, 119.97, 125.79, 126.49, 128.97, 134.07, 143.06, 146.83 (C_2HN_3, $C_6H_4N_3$); MS (ESI): Calcd for $C_{19}H_{26}N_8NaO_5$ $[M+Na]^+$: 469.19; Found m/z: 469.21; Melting point: 144°C-147°C.

Methyl 2-[(*S*)-2-amino-propionamido]-2-deoxy-6-{4-(thiophen-3-yl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (108), (CR III 51).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 3-ethynyl-thiophene and cleaved from the solid phase after Fmoc-cleavage. Compound **108** (16.5 mg, 100% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **108** (7.5 mg, 54%) was obtained as white powder. $[\alpha]_D^{21}$ +9.1 (*c* = 0.53, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 1.52 (d, 3H, CH_3 of Ala), 3.28 (s, 3H, OCH_3), 3.54 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.90 (m, 2H, H-4, CH of Ala), 3.98 (m, 2H, H-2, H-5), 4.25 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.73 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 2H, H-6), 7.50, 7.75 (m, 3H, C_4H_3S), 8.25 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 17.41 (CH_3 of Ala), 49.72 (CH of Ala), 51.60 (C-6), 53.34 (C-2), 57.11 (OCH_3), 69.09 (C-4), 72.67 (C-3), 74.81 (C-5), 102.89 (C-1), 121.30, 122.59, 126.46, 127.63 (6C, C_2HN_3, C_4H_3S); MS (ESI): Calcd for $C_{16}H_{24}N_5O_5$ $[M+H]^+$: 398.15; Found m/z: 398.24; Melting point: 169°C (decomposition).

Methyl 2-[(*S*)-2-amino-propionamido]-2-deoxy-6-{4-(3-trifluoromethyl-phenyl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (109), (CR III 52).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 3-ethynyl-trifluorotoluene and cleaved from the solid phase after Fmoc-cleavage. Compound **109** (16.9 mg, 93% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **109** (8.5 mg, 53%) was obtained as white powder. $[\alpha]_D^{21}$ +15.2 (*c* = 0.50, MeOH); ^1H NMR (500 MHz, CD_3OD): δ 1.52 (d, 3H, CH_3 of Ala), 3.28 (s, 3H, OCH_3), 3.68 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.90

(m, 2H, H-4, CH of Ala), 3.97-4.03 (m, 2H, H-2, H-5), 4.25 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.77 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 2H, H-6), 7.65, 8.08, 8.15 (m, 4H, C_6H_4), 8.45 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 17.71 (CH_3 of Ala), 49.82 (CH of Ala), 51.88 (C-6), 53.54 (C-2), 56.84 (OCH_3), 69.38 (C-4), 72.82 (C-3), 74.89 (C-5), 102.72 (C-1), 122.55, 123.90, 125.65, 129.56, 130.23 (8C, C_2HN_3, $C_7H_4F_3$); MS (ESI): Calcd for $C_{19}H_{25}F_3N_5O_5$ $[M+Na]^+$: 460.18; Found m/z: 460.21; Melting point: 128°C-131°C.

Methyl 2-[(*S*)-2-amino-propionamido]-2-deoxy-6-{4-(4-methyl phenyl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (110), (CR III 55).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-alanine, reacted with 4-etynyl-toluene and cleaved from the solid phase after Fmoc-cleavage. Compound **110** (14.6 mg, 100% recovery, ≥98% purity) was obtainedas the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **110** (9 mg, 63%) was obtained as white powder. $[\alpha]_D^{21}$ +13.0 (*c* = 0.42, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.52 (d, 3H, CH_3 of Ala), 2.36 (s, 3H, CH_3), 3.28 (s, 3H, OCH_3), 3.67 (m, 1H, H-3), 3.89 (m, 2H, H-4, CH of Ala), 3.99 (m, 2H, H-2, H-5), 4.25 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.74 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 2H, H-6), 7.25, 7.68 (m, 4H, C_6H_4), 8.28 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 17.63 (CH_3 of Ala), 21.16 (CH_3), 50.20 (CH of Ala), 51.68 (C-6), 53.69 (C-2), 56.78 (OCH_3), 69.02 (C-4), 72.92 (C-3), 74.93 (C-5), 102.76 (C-1), 122.57, 126.59, 130.04 (8C, C_2HN_3, C_6H_4); MS (ESI): Calcd for $C_{19}H_{27}N_5NaO_5$ $[M+Na]^+$: 428.19; Found m/z: 428.24; Melting point: 143°C-147°C.

Methyl 2-[(*S*)-2-amino-3-methyl-butyrylamido]-2-deoxy-6-{4-(4-methyl phenyl)-[1,2,3]-triazol-1-yl}-β-D-galactopyranoside (111), (CR III 56).

According to the general procedures for solid phase synthesis **93a/b** was loaded, deprotected, coupled with Fmoc-valine, reacted with 4-ethynyl-toluene and cleaved from the solid phase after Fmoc-cleavage. Compound **111** (18 mg, 100% recovery, ≥98% purity) was obtained as the acetate salt. After purification and a final lyophilization from dioxane/H_2O (1:1) **111** was obtained as white powder. ^{1}H NMR (500 MHz, CD_3OD): δ 1.08 (m, 6H, $CH(C\mathbf{H}_3)_2$), 2.19 (m, 1H, $C\mathbf{H}(CH_3)_2$), 2.36 (s, 3H, CH_3), 3.29 (s, 3H, OCH_3), 3.61 (d, *J* = 5.4 Hz, 1H, $CHNH_2$), 3.70 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.1 Hz, 1H, H-3), 3.86 (d, $J_{3,4}$ = 2.9 Hz, 1H, H-4), 3.97 (m, 2H, H-2, H-5), 4.31 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.74 (d, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 2H, H-6), 7.25, 7.69 (m, 4H,

C_6H_4), 8.35 (s, 1H, C_2HN_3); ^{13}C NMR (125 MHz, CD_3OD): δ 18.61 (2C, $CH(\mathbf{C}H_3)_2$), 21.40 (CH_3), 51.81 (C-6), 54.08 (C-2), 56.88 (OCH_3), 59.50 ($CHNH_2$), 69.63 (C-4), 72.95 (C-3), 75.05 (C-5), 102.83 (C-1), 122.56, 126.49, 130.24 (8C, C_2HN_3, C_6H_4); MS (ESI): Calcd for $C_{21}H_{31}N_5NaO_5$ $[M+Na]^+$: 434.24; Found m/z: 434.26; Melting point: 141°C-145°C.

Methyl 2-[(*S*)-2-amino-propionamido]-6-azido-2-deoxy-β-D-galactopyranoside (99), (CR III 40).

93a/b was loaded to the solid support and the phthalimido group cleaved according to the general procedures for solid phase synthesis. Fmoc-alanine was coupled by 3 alternative parallel methods at r.t. After Fmoc-cleavage **99** was cleaved from the solid phase and purified according to the general procedures for solid phase synthesis. Purification and a final lyophilization from dioxane/H_2O (1:1) were done with all three combined batches. Compound **99** (16 mg, 53%) was obtained as white powder.

Method A for amino acid coupling:

According to the general procedure described above for solid phase synthesis, **99** was obtained (9.9 mg, 81% recovery, ≥ 98%) as the acetate salt.

Method B for amino acid coupling:

A stock solution of Ala-Fmoc (4 eq x 35 μmol x 1.5 lantern) and HOBt (4 eq x 35 μmol x 1.5 lantern) in DMF (750 μL) was freshly prepared. In a 96-well plate the freshly prepared stock solution (500 μL) and DIC (4 eq, 21.7 μL) were combined. After 2 min of pre-activation the lantern was added. Five rounds of coupling were performed, each time fresh coupling solutions were used. The first coupling was run overnight. The next three cycles were run for 4 h each and the last one overnight. Compound **99** was obtained (11.6 mg, 95% recovery, ≥ 98%) as the acetate salt.

Method C for amino acid coupling:

Two stock solutions were freshly prepared. Solution **A** consisted of Fmoc-alanine (5 eq x 35 μmol x 1.5 lantern) and HOBt (5 eq x 35 μmol x 1.5 lantern) in DMF (375 μL). Solution **B** consisted of PyBOP (4.9 eq x 35 μmol x 1.5 lantern) in DMF (375 μL). In a 96-well plate 250 μL of stock solution **A**, 250 μL of stock solution **B,** and DIPEA (10 eq, 59.9 μL) were combined. After 2 min of pre-activation the lantern was added. Five rounds of coupling were performed, each time fresh coupling solutions were used. The first coupling was run overnight. The next three cycles were run for 4 h

each and the final one overnight. Compound **99** was obtained (9.9 mg, 81% recovery, ≥ 98%) as the acetate salt $[\alpha]_D^{21}$ -13.9 (*c* = 0.23, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 1.53 (d, 3H, CH_3 of Ala), 3.24 (m, 1H, H-6a), 3.47 (s, 3H, OCH_3), 3.64-3.72 (m, 3H, H-3, H-5, H-6b), 3.76 (d, $J_{3,4}$ = 2.9 Hz, 1H, H-4), 3.93 (m, 2H, H-2, CH of Ala), 4.36 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1); ^{13}C NMR (125 MHz, CD_3OD): δ 18.20 (CH_3 of Ala), 50.93 (CH of Ala), 52.96 (C-6), 54.58 (C-2), 57.41 (OCH_3), 70.66 (C-4), 73.19 (C-3), 76.42 (C-5), 104.01 (C-1), MS (ESI): Calcd for $C_{10}H_{19}N_5NaO_5$ $[M+Na]^+$: 312.13; Found m/z: 312.23; Melting point: 145°C (decomposition).

Other tests performed

3-(2-Hydroxyethyl-sulfanyl)-cyclohexanone (14), (CRV 7).

2-Cyclohexen-1-one (56 μL, 0.58 mmol) was dissolved under argon in degassed DCM (3 mL). Freshly distilled and degassed diethylamine (1.6 mL) was added followed by 2-mercaptoethanol (27 μL, 0.38 mmol). The reaction was stirred at r.t. for 6 h and then concentrated under reduced pressure. The resulting material was purified by silica gel chromatography (PE/EA 1:1) to obtain **14** (48 mg, 72%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.70, 2.10, 2.25-2.38 (m, 7H, C_6H_9), 2.54 (s, 1H, OH), 2.70 (m, 3H, CH_2S, 1H of C_6H_9), 3.09 (m, 1H, CH), 3.70 (m, 2H, C**H**$_2$OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.10, 31.67 (2C, 2 CH_2 of C_6H_9), 33.55 (CH_2S), 40.88 (CH_2 of C_6H_9), 42.73 (CH), 48.18 (CH_2 of C_6H_9), 61.06 (CH_2OH), 208.96 (CO).

Annex 1: Data extracted from the literature

In this section, the data extracted from the literature are summarized. Inhibition values determined for various monovalent monosaccharides, as well as natural ligands, for different mammalian hepatic lectins are presented. The binding assays performed were usually based on ^{125}I-marked natural ligands, which were put to compete with different concentrations of the inhibitors under study. For each case the assays are shortly described. This data was of high relevance for the basis of the present work. Each publication is presented separately and in chronological order.

1. Extracted from: J.U. Baezinger, Y. Maynard, *J. Biol. Chem.* **1980**, *255*, 4607-4613.

Data reported: Preliminary inhibitory studies in human hepatic lectin.

Table I: Inhibition constants for binding by the human hepatic lectin

	Gal	**Gal*N*Ac**	**ki**
	Residues /mol of protein or glycopeptide		[M]
Asialo-orosomucoid	20	0	1.7x10-9
Asialofetuin	12	3	1.7x10-8
Asialoceruloplasmin	12	0	8.6x10-8
Asialotransferrin	5	0	3.3x10-6
Lactose	1		2.2x10-4
β-Methylgalactoside	1		2.5x10-4
α-Methylgalactoside	1		2.7x10-4

$$ki = \frac{[i]}{\left[\frac{k_d{}^i}{k_d} - 1\right]}$$

$[i]$ molar concentration of inhibitor.

k_d dissociation constant in absence of the inhibitor

$k_d{}^i$ dissociation constant in presence of the inhibitor

ki values established by carrying out binding assays with increasing amounts of ^{125}I-asialo-orosomucoid in the presence of a known amount of inhibitory ligand

Assay description:

A modification of the assay A described by Hudgin *et al*.[13] was used. Aliquots of crude Triton X-100 extracts, affinity column fractions, or purified protein containing 0.5 – 5.0 μg of the hepatic lectin, were incubated for 30 min at 25 °C with 360 ng of ^{125}I-asialo-orosomucoid in 0.5 mL or 50 mM Tris-HCl, pH 7.8, 40mM $CaCl_2$, 1M NaCl and 0.2% Triton X-100. The lectin-^{125}I-asialo-orosomucoid complexes were then precipitated by addition of saturated ammonium sulfate, which had been adjusted to pH 7.8 with solid Tris, and chilled for 10 min on ice. The precipitates were collected on glass fiber filters and washed three times with a cold 50% saturated ammonium sulfate solution containing 10 mM $CaCl_2$. Blanks consisted of the same incubation mixture, but without binding protein. Under the conditions used, 1 μg of human carbohydrate binding protein bound approximately 45-50 ng of asialo-orosomucoid.

2. Extracted from: D. T. Connolly, R. R. Townsend, K. Kawaguchi, W. R. Bell, Y. C. Lee, *J. Biol. Chem*. **1982**, *257*, 939-945.

Data reported: Inhibitory action of glycosides towards the rabbit receptor.

Table: Inhibition of ^{125}I-asialoorosmucoid binding to isolated hepatic lectin and to rabbit hepatocytes by various glycosides and monosaccharides

Inhibitors	**Isolated lectin IC_{50} (μM)**	**Hepatocytes IC_{50} (μM)**
Asialo-orosomucoid	0.005	0.003
Aminohexyl β-D-Gal	280	300
Aminohexyl β-D-thio-Gal	380	400
Aminohexyl α-D-thio-Gal	150	250
p-Nitrophenyl β-D-thio-Gal	200	500
Methyl β-D-Gal	1000	1000
Methyl β-D-thio-Gal	1300	1000
Methyl α-D-Gal	1600	1900
Gal*N*Ac	90	70
Lactose	300	400
Gal	1700	1900
Glc	60.000	90.00
Man	30.000	>100.000
Glc*N*Ac	300.000	>100.000

Assay description:

a) Binding assays using the purified hepatic lectin were based on assay A of Hudgin *et al*.[13]

The lectin (90ng) was incubated with ^{125}I-asialo-orosomucoid (5 ng, 5 x 10^5 cpm), and varying amounts of inhibitors for 30 min at 25 °C in 0.5 mL of buffer (0.05 M Tris, pH 7.8, 1M NaCl, 0.05 M $CaCl_2$, 0.6% BSA, and 0.5% Triton X-100). Bound ^{125}I-asialo-orosomucoid was determined by ammonium sulfate precipitation followed by filtration. Nonspecific binding to the filter was corrected for by subtracting the amount of ^{125}I-asialo-orosomucoid retained on the filter in the absence of lectin.

b) Binding assays using hepatocytes in suspension culture.

Were carried out in capped 12 x 75 mm polystyrene tubes, which were rotated vertically (4 rpm) at 0-1 °C. The reaction mixtures contained the inhibitors to be tested, ^{125}I-asialo-orosomucoid (5 ng, 5 x 10^5 cpm), and 2.5 x 10^6 rabbit hepatocytes in 1.0 mL of modified Dulbecco's Eagle medium. After 100 min incubation, 100 μL sample were removed and added to 400 μL microfuge tubes containing 100 μL of a 4:1 (v/v) mixture of silicon oil and light mineral oil. After centrifuging for 10-12 s in an Eppendorf centrifuge, the aqueous upper layer was removed by aspiration. The microfuge tubes were washed twice more before removing most of the oil layer. The cell pellet remaining was counted for radioactivity. Nonspecific binding was corrected for by subtracting the amount of ^{125}I-asialo-orosomucoid that became cell associated under the above conditions but in the presence of 8 mM EGTA.

3. Extracted from: R.T. Lee, R. W. Myers, Y.C. Lee, *Biochemistry* **1982**, *21*, 6292-6298.

Data reported: Binding characteristics of rabbit liver Gal/Gal*N*Ac-specific lectin

Table I: IC_{50} values of **analogues of Methyl α-D-Galactopyranoside** having different substituents at **C-5**

C-5 substituent	IC_{50} (mM)	C-5 substituent	IC_{50} (mM)
CH_2OH (Gal)[a]	1.6	H (L-Ara)	12
CH_2-H(D-Fuc)	1.6	CO_2Me	16
CH_2N_3	1.2	$CONH_2$	19
CH2Br	1.1	$CONHNH_2$	9
CH_2NH_2	4.3	CO_2H	~200
CH_2NH_2[b]	1.9		
CH_2OTs[c]	1.0		

[a] The IC_{50} value is from Connolly *et al*.[34] [b] Methyl glycoside of β configuration. [C] Due to insufficient solubility of the methyl glycoside, the reducing sugar (6-O-tosyl-D-galactose) was used.

Table II: IC_{50} values of various **β-Glycosides of D-galactopyranose and D-glucopyranose**

aglycon	Glc (IC_{50} mM)	Gal (IC_{50} mM)
none	16	1.7[b]
OCH_3[c]	26	1.6[b]
OCH_3	17	1.0[b]
$OCH_2CH=CH_2$	12	0.47
$O(CH_2)_6NH_2$		0.28
SCH_2CO_2H	12	2.0
SCH_2CN	2.0	0.13
$SCH_2C(=NH)NH_2$	0.8	0.3
OPh	4.0	
$OC_6H_4NO_2$ (para)	1.7	
$SCH_2CONHCH_2CO_2Et$	2.5	0.7
$SCH_2CONHCH_2CO_2H$	2.1	
$SCH_2C(=NH)NHCH_2CO_2H$	0.75	0.6
$SCH_2CONHCH_2CH(OMe)_2$	2.0	0.75
$S(CH_2)_5CONHCH_2CH(OMe)_2$	4.3	0.23
(1,4)Glc	2.0 (cellobiose)	0.3 (lactose)
(1,4)Glc[c]	2.6 (maltose)	
(1,4)GlcSCH_2CN	1.5 (cyanomethyl thiocellobioside)	

[a] Name of the compound is given in parentheses. [b] Data from Connolly *et al*.[34]

[c] α-glycoside

Table III: IC_{50} values of miscellaneous sugars and glycosides

Compound	IC_{50} mM
Galβ(1→3)GlcNAc	0.9
Galβ(1→4)GlcNAc	0.8
Galβ(1→6)GlcNAc	0.3
Galβ(1→3)GlcNAcO$(CH_2)_6NH_2$ (β)	0.2
Galβ(1→4)GlcNAcO$(CH_2)_6NH_2$ (β)	0.4
Galβ(1→6)GlcNAcO$(CH_2)_6NH_2$ (β)	0.5
Galβ(1→6)Glc	0.9
Galα(1→6)Glc	2.8
GlcNAcβ(1→6)Gal	2.8
GlcNAcβ(1→6)Gal-$SCH_2CONHCH_2CH(OMe)_2$(β)	0.6
N-acetylgalactosamine[a]	0.09
Me 2-*O*-Ac-α-Gal	0.04
1-cyano-1-deoxy.Gal (β)	1.0
1-carboxyl-1-deoxy.Gal (β)	2.5
Me 3,6-anhydro-α-Gal	>>180[b]
Me 4,6-*O*-benzylidene-α-Gal	>>20[b]

[a] From Connolly *et al*.[34] [b] Not inhibitory at the concentration shown

Assay description:

Based on assay A of Hudgin *et al*.[13]

An inhibitor (usually in the concentration range of 5 x 10^{-2} to 5 x 10^{-6} M) and ^{125}I-asialo-orosomucoid (approx. 6 x 10^{-10} M) were incubated with the lectin (approx. 4 x 10^{-10} M) in a total volume of 0.5 mL at pH 7.8 in the presence of 0.05 M $CaCl_2$, 0.6% BSA, and 0.5% Triton X-100 for 0.5 h at 25 °C. The lectin was precipitated with 45% ammonium sulfate and collected by filtration through a glass fiber disk. Radioactivity on the disk was counted to determine the amount of ^{125}I-asialo-orosomucoid bound to the lectin.

4. Extracted from: R.T. Lee, Y.C. Lee, *Biochemistry* **1986**, *25*, 6835-6841.

Data reported: Inhibitory studies in isolated hepatic lectin from rabbit or rat hepatocytes.

Table I: Binding affinity of glycopeptide derivatives.

Stages of Gal modification	Ki for purified rabbit lectin (μM)	Ki for rat hepatocytes (μM)
none	2	90
6-oxo	120	Not inhibitory at 100μM
6-(benzylamino)-6-deoxy	0.9	280
6-amino-6-deoxy	0.9	270
6-(4-azidobenzoamido)-6-deoxy	1.4	430

Assay description:

The inhibition assay was based on assay A of Hudgin et al[13], and carried out according to Connolly *et al*.[34]

5. Extracted from: T.C. Wong, R.R. Townsend, Y.C. Lee, *Carbohydrate Research* **1987**, *170*, 27-46.

Data reported: Binding studies using isolated rat hepatocytes with synthetical D-Galactosamine derivatives.

Modifications on:

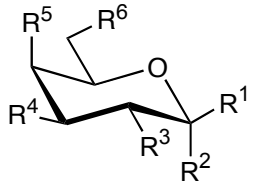


Table I: Inhibition results showing effect of changes in *N*-substitution.

R^1 (β)	**R^3**	**IC_{50} (mM)**	**Relative potency**
OCH_2CH_2OH	NHAc ($NHCOCH_3$)	0.2	1
OCH_2CH_2OH	$NHCOCH_2CH_3$	0.2	1
OCH_2CH_2OH	$NHCOC_6H_5$	0.8	0.25
OCH_2CH_2OH	NPhth	8.0	0.025
$O(CH_2)_6NH_2$	$NHCOCF_3$	0.2	1
$O(CH_2)_6NH_2$	NHAc	0.06[a]	3.333

[a] This result was personal communication from R.T. Lee, and has been adjusted according to the potency of Gal*N*Ac of the authors and her experiment.

Table II: Inhibition results showing effect of changes of hydroxyl groups.

R^1 (β)	R^2 (α)	R^3	R^4	R^5	IC_{50} (mM)	Relative potency
$OCH_2CH=CH_2$	H	OH	OH	OH	0.2	1
$OCH_2CH=CH_2$	H	OH	OH	OCH_3	0.08	2.5
$OCH_2CH=CH_2$	H	OCH_3	OH	OH	70	0.0029
$OCH_2CH=CH_2$	H	OH	OCH_3	OCH_3	31	0.0065
H	$OCH_2CH=CH_2$	OH	OH	OH	0.12	1.7
H	$OCH_2CH=CH_2$	OH	F	OH	>1.5	Inactive at 1.5 mM

Assay description:

Freshly isolated rat hepatocytes (0.25 mL) were added to 0.75 mL of medium containing the desired concentrations of inhibitors and 125 pM ^{125}I-asialo-orosomucoid in 1.5 mL polypropylene tubes. After incubation for 2 h at 0-2°C with end-over-end rotation, the total cell-associated radioactivity was determined by centrifuging 200 μL of the cell suspension over 4:1 silicone-mineral oil at 10.000 g for 30 s. The snipped pellets were counted for radioactivity. Total cell-associated radioactivity in the absence of inhibitor was also determined. Nonspecific binding was determined by placing 200 μL of the cell suspension from tubes not containing inhibitor in a tube containing 4 μL of 0.5 M EDTA, pH 7.8 solution and determining after 10 min the cell-associated radioactivity.

6. Extracted from: S.T. Iobst, K. Drickamer, *J. Biol. Chem.* **1996**, *271*, 6686-6693.

Data reported: Competition assays in isolated RHL1-CRD and CRD of macrophage galactose receptor of rat.

Table I: Binding constants of galactosamine derivatives binding to RHL1

Inhibition constants for derivatives of galactosamine acylated on the *N*-2 position were determined using solid phase binding competition assays. The Ki for each sugar derivative relative to the Ki for Gal*N*Ac is reported.

Sugar derivative	**Acyl Chain (on *N*-2)**	**Ki/Ki_{GalNAc}**
N-formylgalactosamine	-COH	13 ±1
N-acetylgalactosamine	$-COCH_3$	1
N-propionylgalactosamine	$-COCH_2CH_3$	0.13 ± 0.04
N-n-butanoylgalactosamine	$-COCH_2CH_2CH_3$	0.29 ± 0.06
N-iso-butanoylgalactosamine	$-COCH(CH_3)_2$	0.82 ± 0.06

Ki: concentration of monosaccharide giving 50% inhibition of neoglycoprotein binding according to the equation: Bound radioactivity= MAX/(Ki+ [monosaccharide]). MAX is the total amount of radioactivity bound in the absence of competing monosaccharide.

Table II: Dissociation constants and relative inhibition constants were determined using the solid phase binding assay. Ki values for Gal*N*Ac relative to the Ki for Gal are reported along with dissociation constants for Gal_{34}-BSA.

CRD	$Ki_{Gal}/Ki_{,GalNAc}$	K_D
MGR	1.2 ± 0.4	90 ± 40
RHL1	60 ± 8	70 ± 10

K_D : concentration of the ligand at which half-maximal specific saturable binding is achieved

Assay description:

a) Competitive binding assay

The protein was immobilized on polystyrene wells. The coating was performed overnight at 4°C with 50 μL/well CRD at a concentration of approximately 50 ng/mL in loading buffer. The protein was removed, and the wells were rinsed in salt solution (1.25 M NaCl, 25 mM Tris-Cl, pH 7.8). After rinsing, the wells were immediately treated with 5% bovine serum albumin in salt solution for 2 h. This solution was discarded, and the wells were again rinsed in salt solution. Ligand (18.000 cpm/well ^{125}I-Gal_{34}-BSA) in blocking solution containing the appropriate concentration of Ca^{+2} and competing ligands was pippeted into the wells and incubated for 2 h. Wells were rinsed with loading buffer and shaken until excess moisture was removed. Residual radioactivity was counted.[101] Each assay was performed in duplicate. A non linear, least squares fitting program (Sigma Plot, Jandel Scientific) was used to obtain Ki values, corresponding to the concentration of monosaccharide giving 50% inhibition. Mean ± standard deviation values for at least three independent assays were used to calculate Ki values.

b) Direct binding assay

Polystyrene plates were coated with CRD and blocked with BSA as for the competition assay. ^{125}I-Gal_{34}-BSA was diluted with non radioactive Gal_{34}-BSA to a final specific activity of 1-2 x 10^5 cpm/μg. Serial dilutions (2 fold) prepared in BSA-

loading buffer (1.25 M NaCl, 25mM $CaCl_2$, 25 mM Tris-Cl, pH 7.8, containing 5% BSA) were incubated in wells for 2h at 4°C. Wells were emptied, rinsed three times with loading buffer at 4°C, and counted. Assays were performed in duplicate. Values reported are the mean ± standard deviation for three independent assays.

7. Extracted from: D. Yi, R.T. Lee, P. Longo, E. T. Boger, Y.C. Lee, W.A. Petri Jr, R. L. Schnaar, *Glycobiology* **1998**, *8*, 1037-1043.

Data reported: Inhibition studies performed in rat hepatic lectin (lectin not isolated).

Table I: Lectin inhibition by Gal*N*Ac and related derivatives

Inhibitor	**Rat hepatic membranes $IC_{50}(\mu M)$**
Gal*N*Ac	150
Allyl β-Gal*N*Ac	160
Allyl α-Gal*N*Ac	89
Allyl 3-deoxy-β-Gal*N*Ac	(2500)[a]
Allyl 4-deoxy-β-Gal*N*Ac	(2500)[a]
Allyl 6-O-methyl-β-Gal*N*Ac	90
Allyl 4-fluoro-α-Gal*N*Ac	(2500)[a]
N-benzoyl galactosamine	1300
$GalNH_2$	>25.000

[a]Values in parentheses indicate no inhibition at the given value (highest concentration tested)

Assay description:

Binding of ^{125}I-Gal*N*Ac$_{39}$BSA to rat hepatic plasma membranes was performed in a 100 μL reaction containing assay buffer (25 mM Tris-HCl buffer pH7.8, 150 mM NaCl, 20 mM $CaCl_2$, 10 mg/mL BSA, 0.02% Triton X-100), and the indicated concentrations of radioligand, rat liver plasma membranes and saccharide inhibitors. Reactions were incubated with gentle agitation for 90 min at 4°C, then rapidly diluted with assay buffer without BSA and rinsed onto glass fiber filters (presoaked in 25 mM Tris buffer (pH 7.8), 1 mg/mL BSA). Specific radioligand binding was determined by using a radiation counter.

Annex 2: Competitive target-based assay

All curves obtained from the competitive target-based assay used for IC_{50} determination for all the synthesized compounds as well as for references are here presented.

The competitive target-based assay was developed in our institute by Daniela Stokmaier.[50] Assays were run by herself, who also kindly taught me how to run the assay and by her diploma student Katrin Schwingruber. A general description of the procedure can be found here.

Experimental

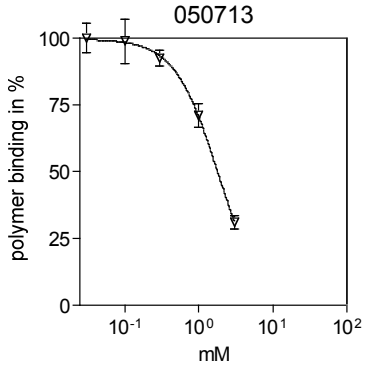
Nunc MaxiSorb 96 well plates were coated with H1-CRD monomer from the same production batch (3 μg/mL in HEPES assay buffer containing 1 mM of Ca^{+2}). Plates were incubated overnight at 4°C. One row contained only buffer to be employed for background and blank. The assay buffer consisted in 20 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$ and with the pH adjusted to 7.4 with NaOH 10 M.

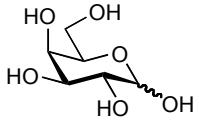
The following day, the coating solution was discarded and the plates were dried by tapping them on several layers of tissue paper. The plates were then incubated with 150 μL/well of blocking buffer for 1 h at 4°C. The blocking buffer consisted in 3% BSA in assay buffer with added 5 mL NaN_3 (10%)/ 500 mL buffer. The plates were then washed with assay buffer (3 x 150 μL/well) tapping plates dry between wash steps. First, inhibitors diluted in assay buffer (2 x concentrated, 50 μL/well) with DMSO were added (see inhibitors solutions), then biotinylated β-Gal*N*Ac-polyacrylamide-polymer and the streptavidin peroxidase complex (0.5 μg/mL, 2 x concentrated, 50 μL/well) was added. The plates were left in the shaker for 2 h at r.t. and were then washed as before. For detection, 100 μL/well of ABTS peroxidase substrate kit was added. After 10 min the colour development was stop by adding 100 μL/well of oxalic acid 2% in H_2O. The optical densitiy (O.D.) was determined at 415 nm on a plate reader.

Inhibitors solutions:

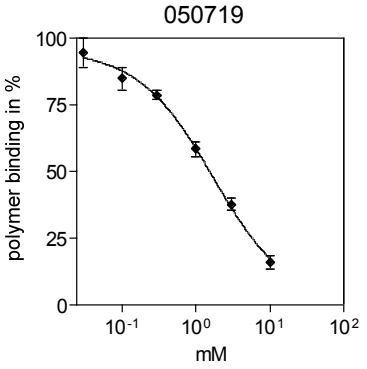
20 mM stock solutions of inhibitors were prepared in DMSO to overcame the problem of solubility of some inhibitors. Dilutions were done with HEPES assay buffer to obtain 20 mM, 6 mM, 2 mM, 0.6 mM, 0.2 mM and lower in the cases required (concentrations on the plate were therefore half of each mentioned concentration). The maximum amount of DMSO on plate was of 5% for the most concentrated inhibitor solution. As in presence of DMSO IC_{50} values were always higher than without it, all compounds and references were measured containing DMSO to obtain comparable results.

1. Reference compounds

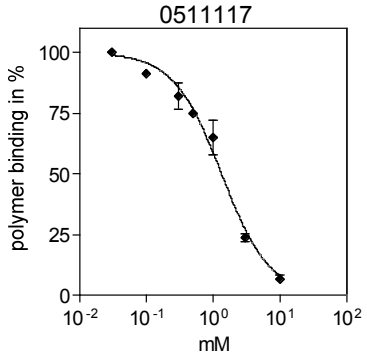


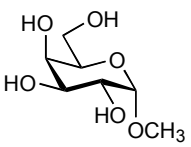


IC_{50}(mM) Galactose: 1.796

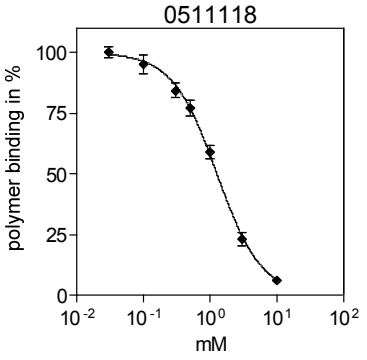


IC_{50}(mM) Galactose in 5% DMSO: 1.713

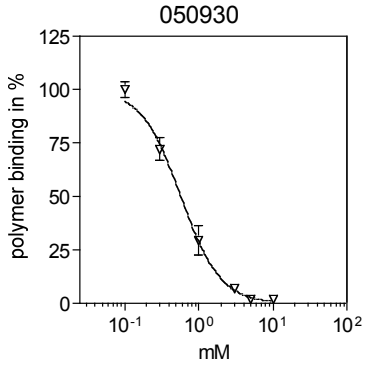


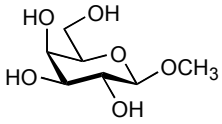


IC_{50}(mM) methyl α-galactopyranoside: 1.315

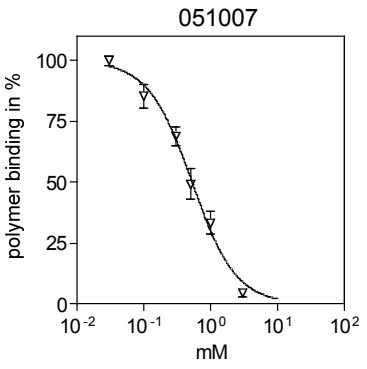


IC_{50}(mM) methyl α-galactopyranoside: 1.245

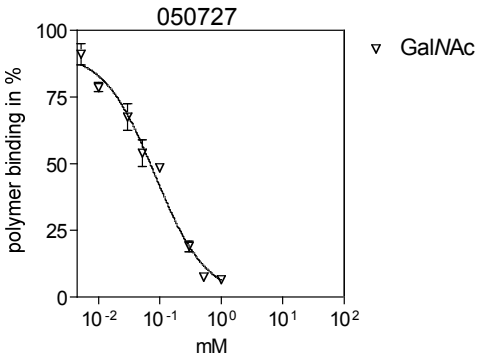




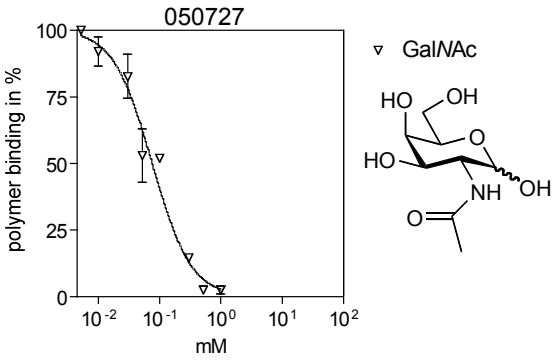
IC_{50}(mM) Methyl-β-gal: 0.5726



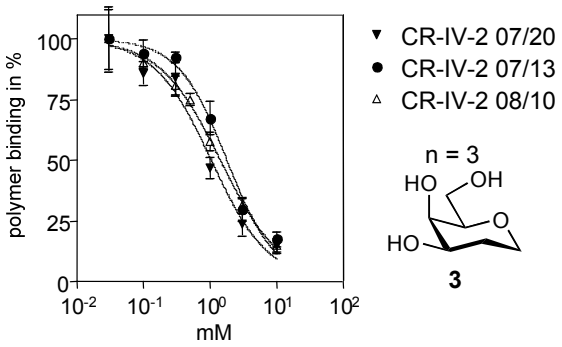
IC_{50}(mM) methyl-β-gal: 0.5174



IC_{50}(mM)	N-Ac-Gal in 5% DMSO
	0.08155

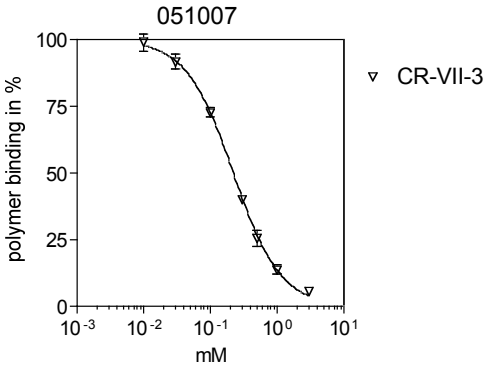


IC_{50}(mM)	N-Ac-Gal in DMSO
	0.07366

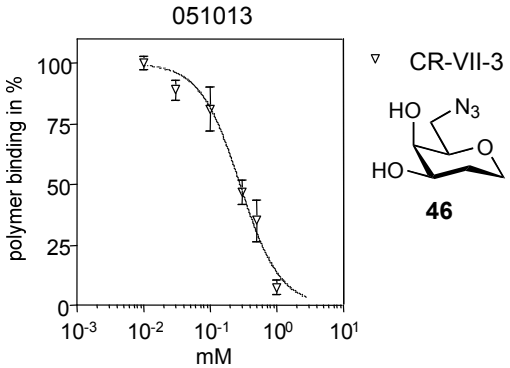


3

IC_{50}(mM)	CR-IV-2 07/20	CR-IV-2 07/13	CR-IV-2 08/10
	1.027	1.764	1.406



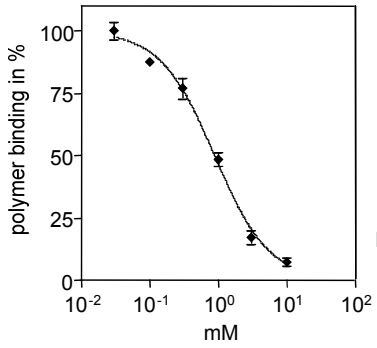
IC_{50}(mM)	CR-VII-3
	0.2184



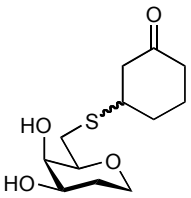
46

IC_{50}(mM)	CR-VII-3
	0.2769

2. Library of galactose mimics based on the *lyxo*-hexitol core (**28** to **39** and **45** and **50**)

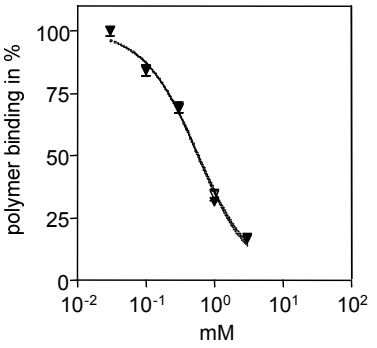


◆ CR-V-8 07/14

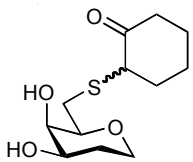


28

IC_{50}(mM)	CR-V-8 07/14
	0.8678

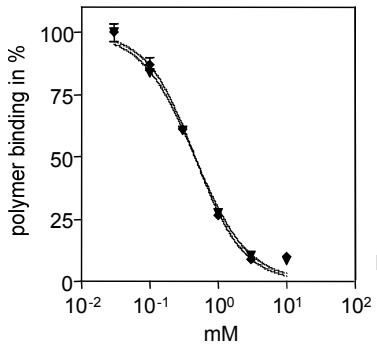


▽ CR-V-16 07/14
▼ CR-V-16 07/20

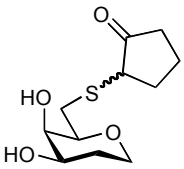


29

IC_{50}(mM)	CR-VI-16	CR-V-16 07/20
	0.5857	0.5640

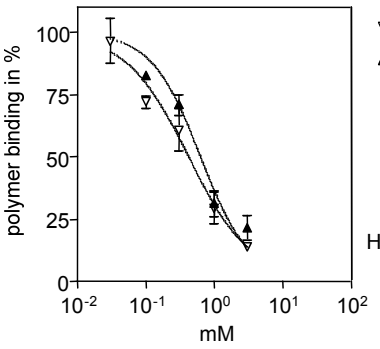


◆ CR-V-12 07/13
▼ CR-V-12 07/20

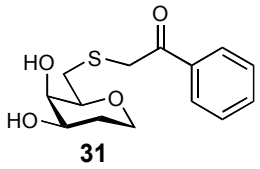


30

IC_{50}(mM)	CR-V-12 07/13	CR-V-12 07/20
	0.4514	0.4476

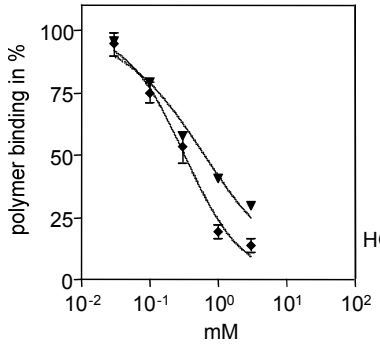


▽ CR-V-20 07/13
▲ CR-V-20 07/20

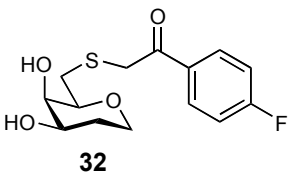


31

CR-V-20 07/13	CR-V-20 07/20
0.4154	0.6230

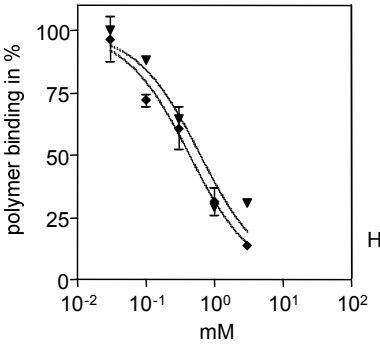


◆ CR-V-21 07/12
▼ CR-V-21 07/20

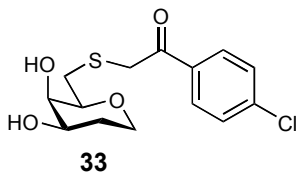


32

IC_{50}(mM)	CR-V-21 07/12	CR-V-21 07/20
	0.3195	0.6244

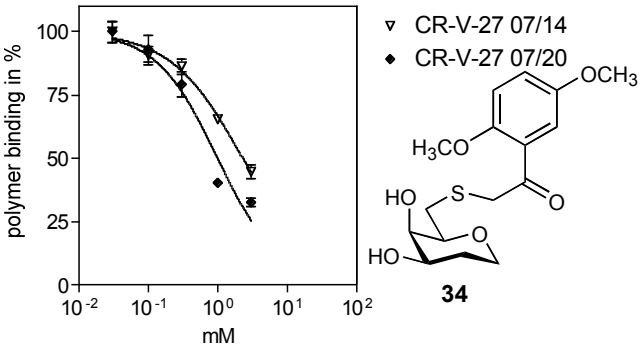


◆ CR-V-22 07/13
▼ CR-V-22 07/20

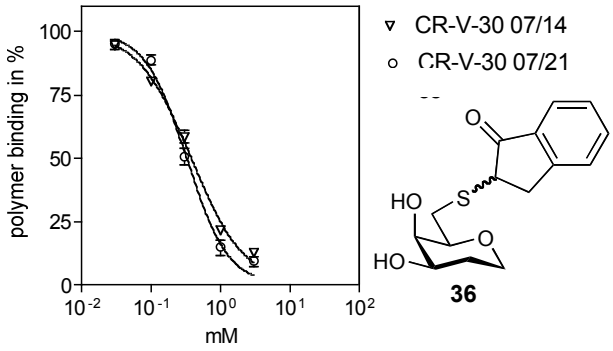


33

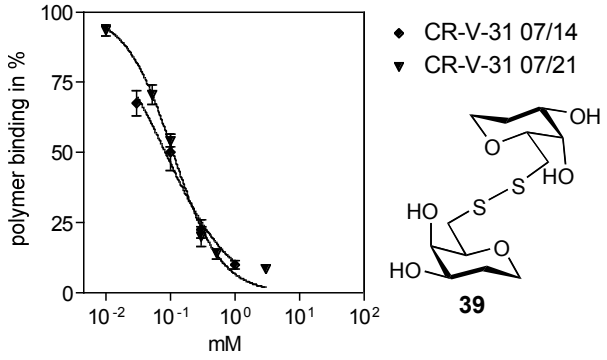
IC_{50}(mM)	CR-V-22 07/13	CR-V-22 07/20
	0.4293	0.6194



IC_{50}(mM)	CR-V-27 07/14	CR-V-27 07/20
	2.259	0.9825

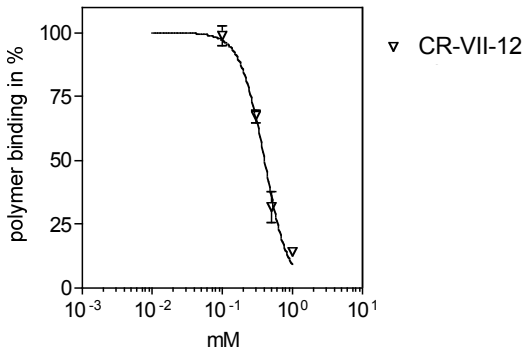


IC_{50}(mM)	CR-V-30 07/14	CR-V-30 07/21
	0.3728	0.3201



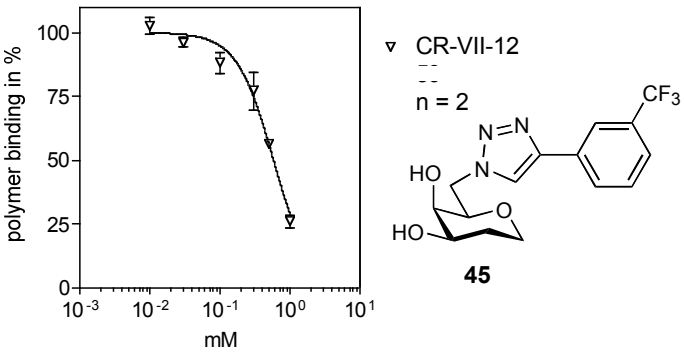
IC_{50}(mM)	CR-V-31 07/14	CR-V-31 07/21
	0.08177	0.1069

051019 CR-VII-12 from stocksol. 19.10.05 STO

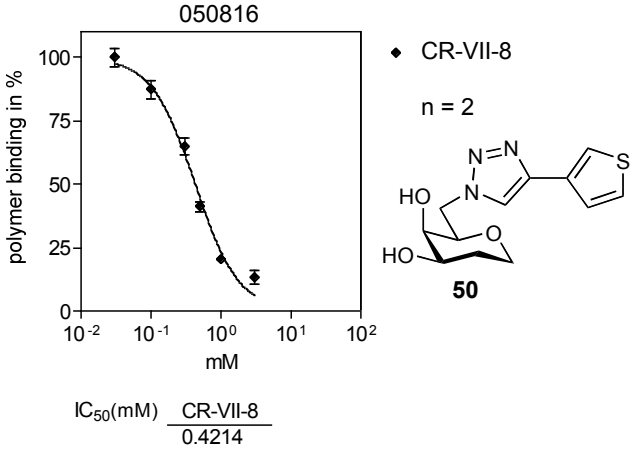


IC_{50}(mM)	CR-VII-12
	0.3912

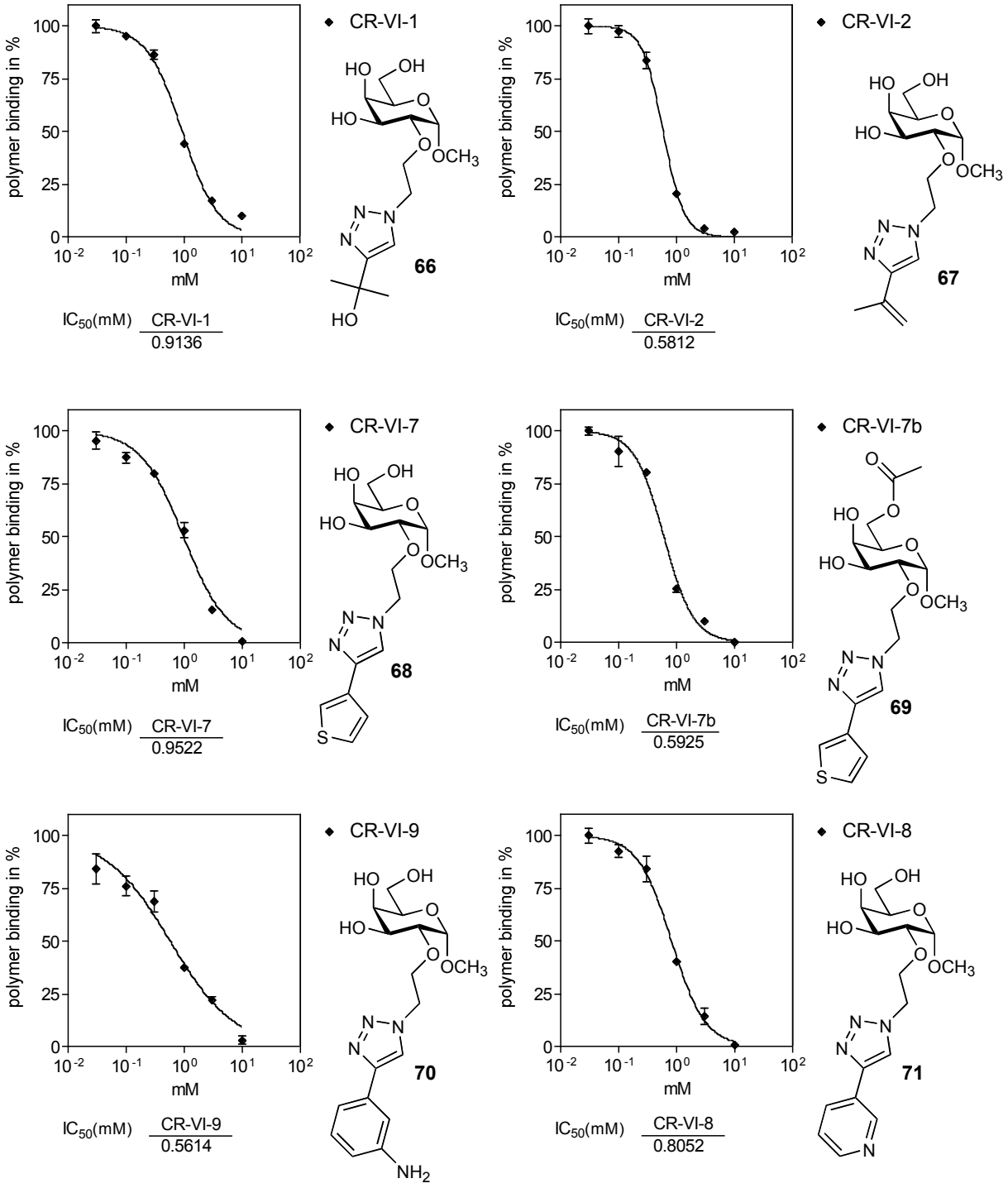
051013 CR-VII-12 from stocksol. 13.10.05 STO

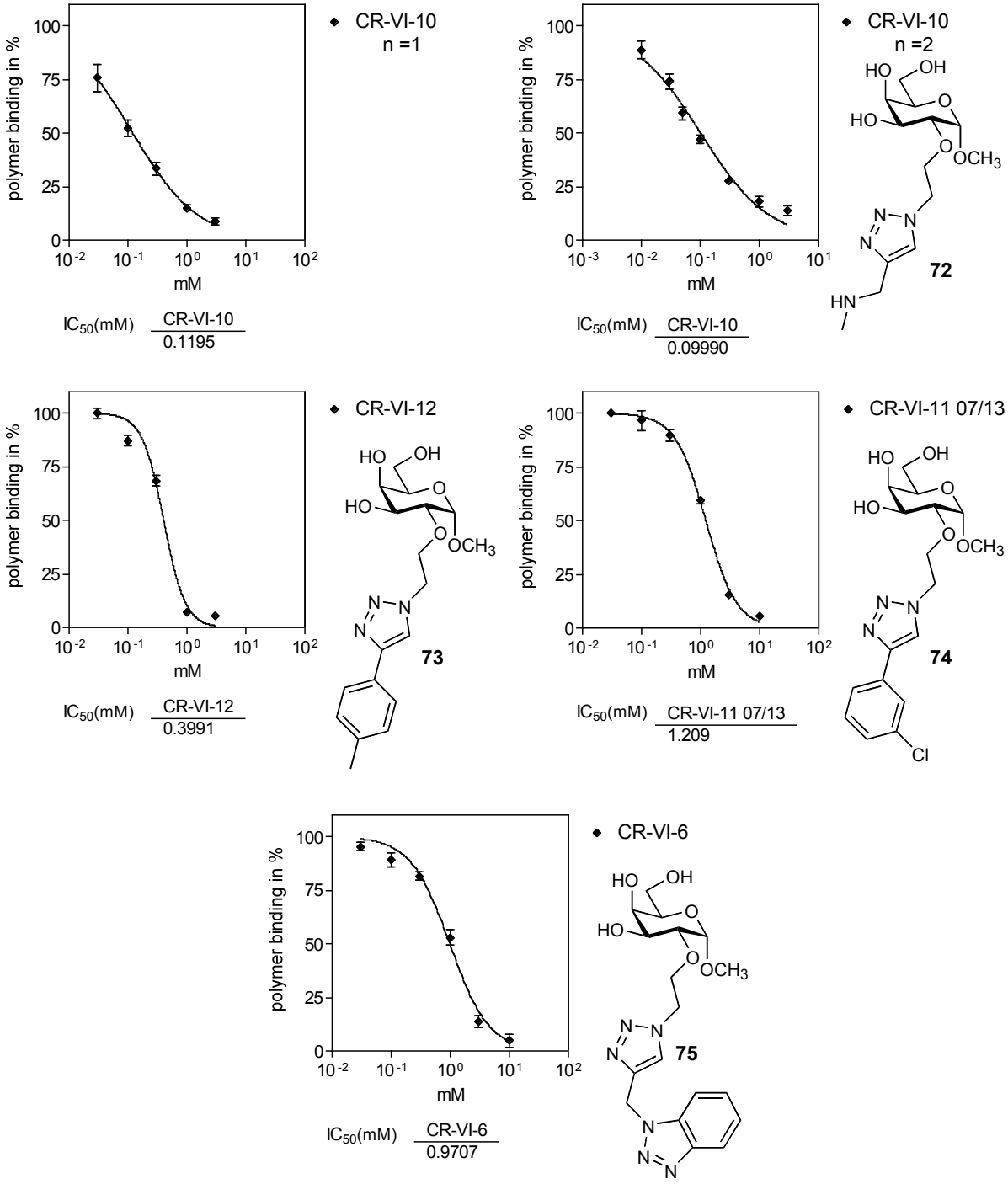


IC_{50}(mM)	CR-VII-12
	0.5712

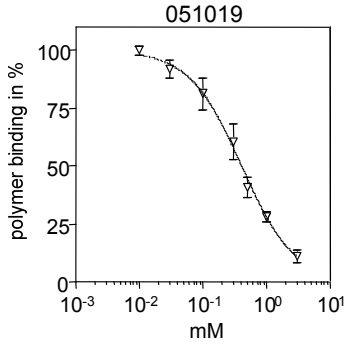

050816
polymer binding in %
100
75
50
25
0
10 -2
10 -1
10 0
10 1
10 2
mM
CR-VII-8
n = 2
N
N
S
HO
O
HO
50
IC50(mM) CR-VII-8
0.4214

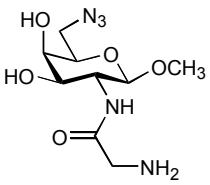
3. Library of methyl α-D-*galacto*-derivatives (**66** to **75**)




polymer binding in %
mM
CR-VI-10
n =1
IC50(mM) CR-VI-10
0.1195
CR-VI-10
n =2
72
IC50(mM) CR-VI-10
0.09990
CR-VI-12
73
IC50(mM) CR-VI-12
0.3991
CR-VI-11 07/13
74
Cl
IC50(mM) CR-VI-11 07/13
1.209
CR-VI-6
75
IC50(mM) CR-VI-6
0.9707

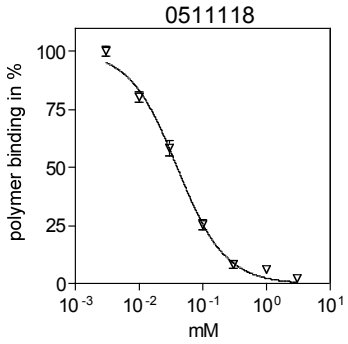
4. Library of methyl β-D-*galacto*-derivatives



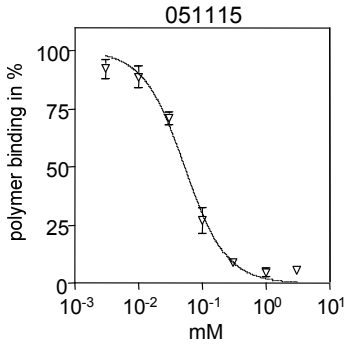


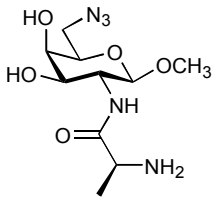
98

$IC_{50}(mM)$ CR-III-57 = 0.4011



$IC_{50}(mM)$ CR-III-40 = 0.03951





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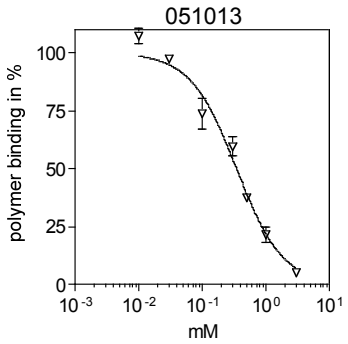
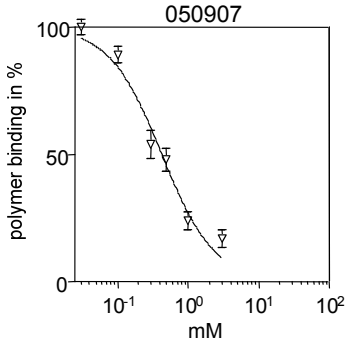
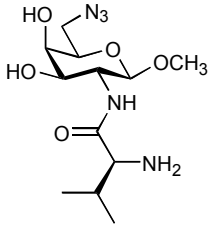
$IC_{50}(mM)$ CR-III-40 = 0.05263



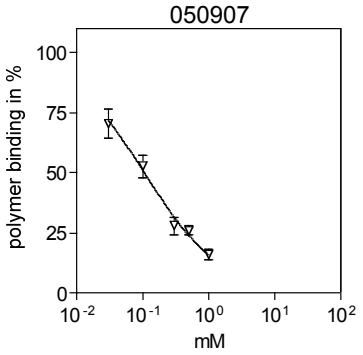
$IC_{50}(mM)$ CR-III-54 = 0.3419



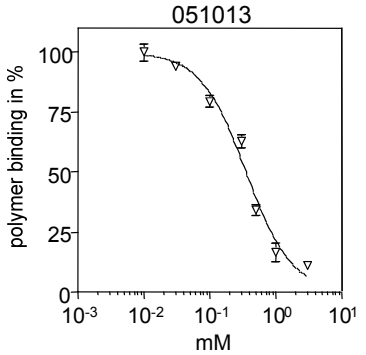


100

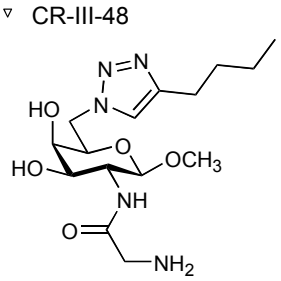
$IC_{50}(mM)$ CR-III-54 = 0.4284



IC_{50}(mM) CR-III-48 = 0.1040



IC_{50}(mM) CR-III-48 = 0.3503



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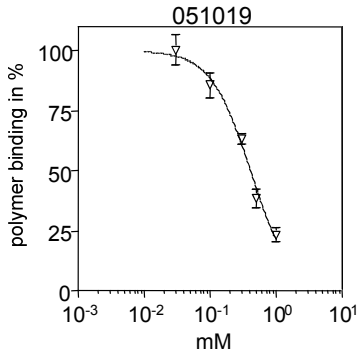
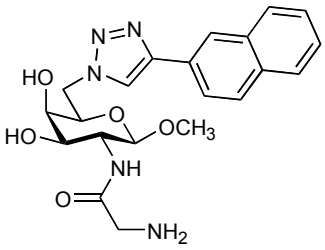


IC_{50}(mM) CR-III-50 = 0.4032



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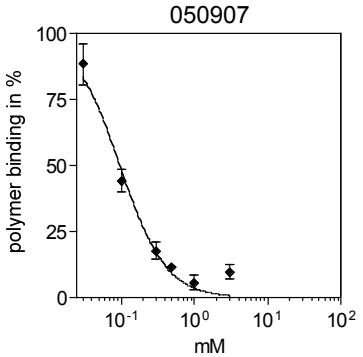
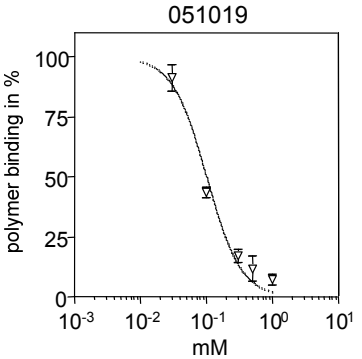
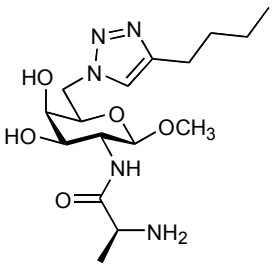


IC_{50}(mM) CR-III-47 = 0.09593



IC_{50}(mM) CR III-47 = 0.09937



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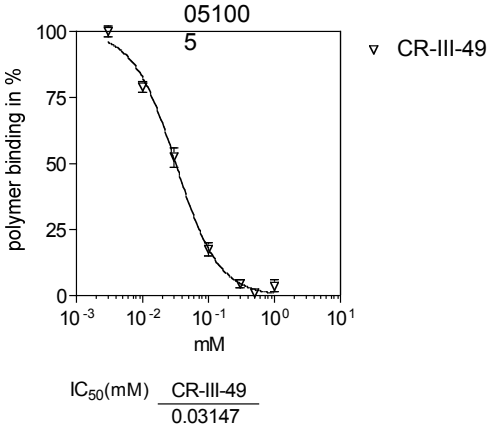
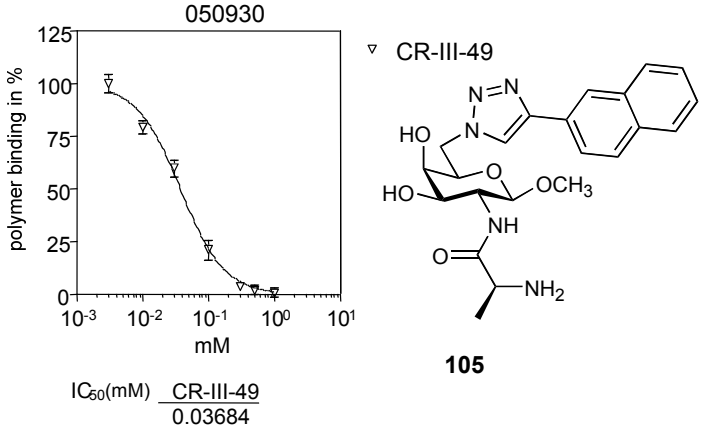
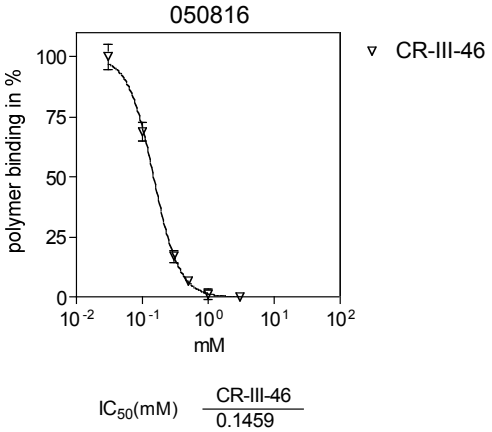
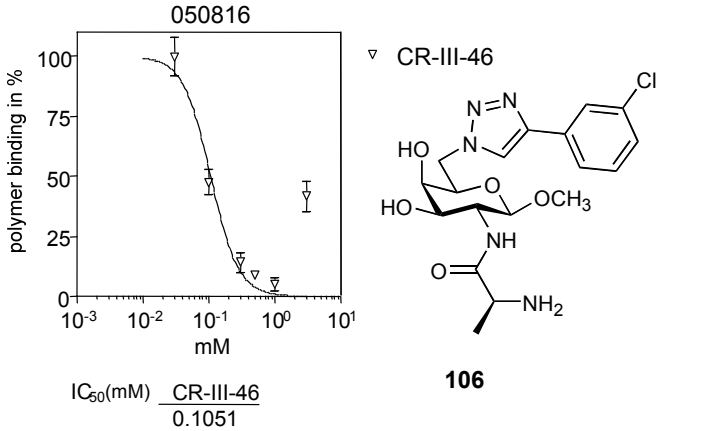

05100
5
CR-III-49
polymer binding in %
mM
IC50(mM) CR-III-49
0.03147

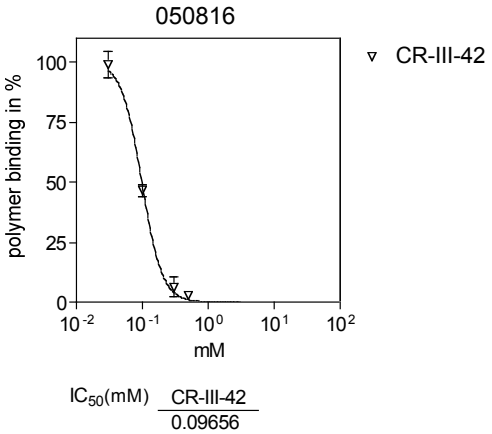

050930
CR-III-49
polymer binding in %
mM
IC50(mM) CR-III-49
0.03684

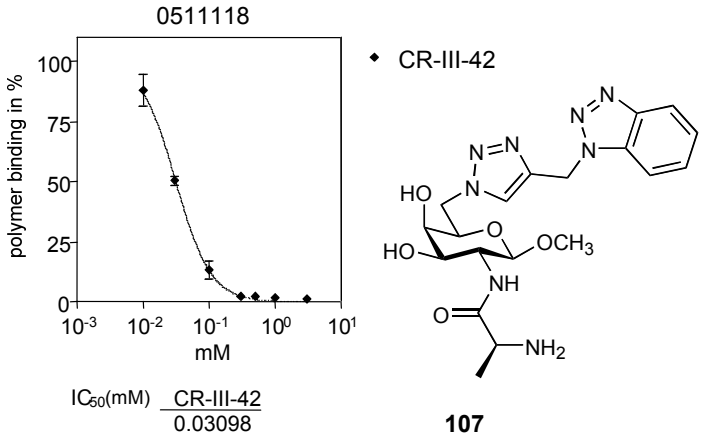
105


050816
CR-III-46
polymer binding in %
mM
IC50(mM) CR-III-46
0.1459

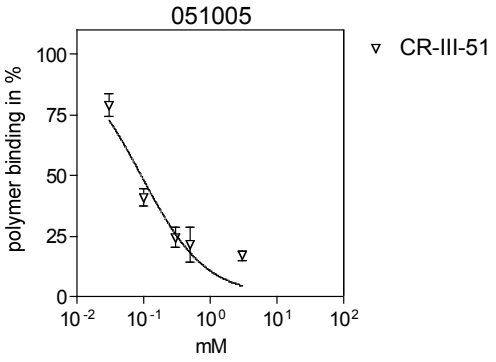

050816
CR-III-46
polymer binding in %
mM
IC50(mM) CR-III-46
0.1051

106

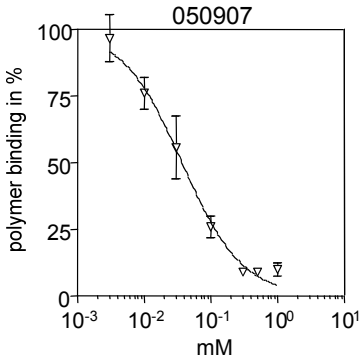

050816
CR-III-42
polymer binding in %
mM
IC50(mM) CR-III-42
0.09656

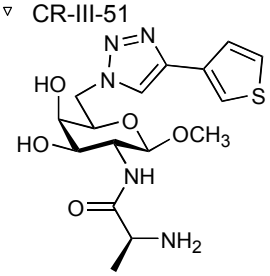

0511118
CR-III-42
polymer binding in %
mM
IC50(mM) CR-III-42
0.03098

107



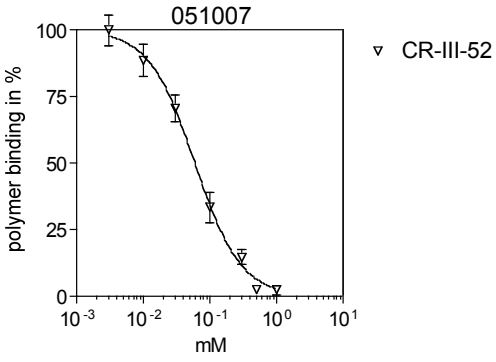
IC_{50}(mM)	CR-III-51
	0.09003



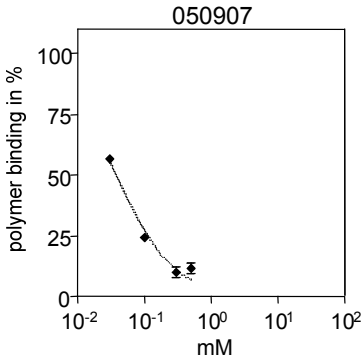


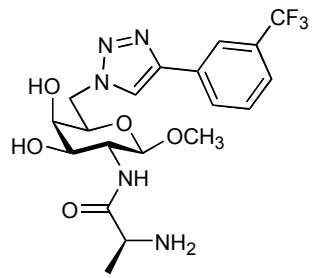
108

IC_{50}(mM)	CR-III-51
	0.03649



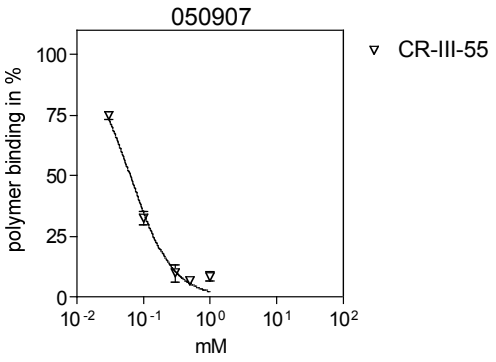
IC_{50}(mM)	CR-III-52
	0.05876



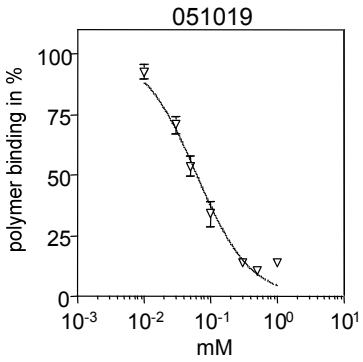


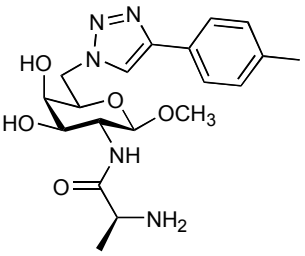
109

IC_{50}(mM)	CR-III-52
	0.03722



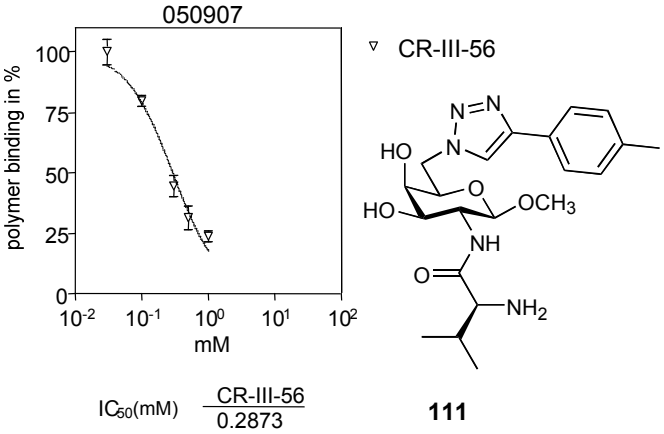
IC_{50}(mM)	CR-III-55
	0.06221





110

IC_{50}(mM)	CR-III-55
	0.06167



$IC_{50}(mM)$ $\frac{\text{CR-III-56}}{0.2873}$

111

Annex 3: Biacore experiments

Surface plasmon resonance data obtained for the compounds analyzed can be found in this section. Biacore experiments were run in our institute, Institute of Molecular Pharmacy of the University of Basel, by Dr. D. Ricklin.

Experimental

All Biacore experiments were performed at 25°C on a Biacore 3000 instrument (Biacore AB, Uppsala, Sweden) equipped with research-grade CM5 sensor chips. Monomeric H1-CRD was covalently immobilized using a standard amine coupling protocol.[102] For this purpose, a single flow cell of the sensor chip was activated by injecting a 1:1 mixture of 0.1 M *N*-hydroxysuccinimide (NHS) and 0.4 M 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) for 10 min at a flow rate of 5 µl/min. Purified H1-CRD in 10 mM HEPES, 2 mM EDTA, pH 7.4 was diluted in 10 mM sodium acetate buffer pH 4.5 to a concentration of approximately 10-50 µg/ml and was injected for 10 min. Finally, the flow cell was deactivated by an injection of 1 M ethanolamine for 10 min. 10 mM HEPES, 50 mM $CaCl_2$, pH 7.4 was used as a running buffer. The immobilization density was determined as the signal increase between the baseline after activation and after the final deactivation step. An untreated flow cell was used as a reference cell in order to eliminate artificial signals from non-specific binding and instrument noise. Data acquisition was done with *BIAcontrol* software (version 3.1; Biacore AB, Uppsala, Sweden) and the raw data were processed with *Scrubber* (version 1.1g; BioLogic Pty., Australia).

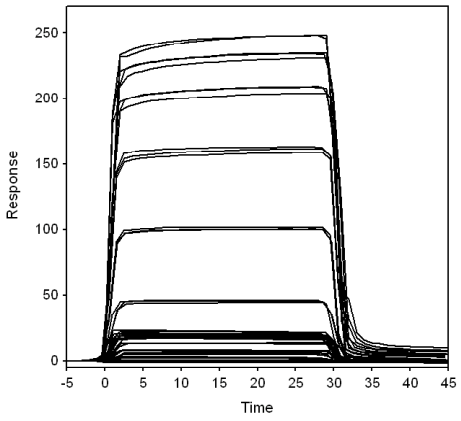
Compound ranking

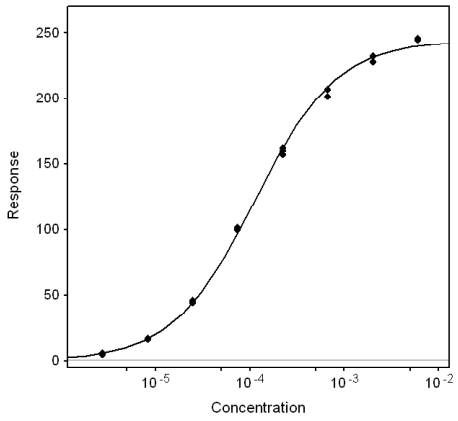
5% DMSO were added to the running buffer for the ranking experiment. Sample solutions were prepared by diluting 200 mM stock solutions in DMSO of each compound in DMSO-free buffer to a sample concentration of 10 mM in 5% DMSO. These solutions were further diluted in running buffer, resulting in 1 mM sample solutions. Each sample was injected for 60 s at a flow rate of 20 µl/min, followed by a undisturbed dissociation phase of 60 s, except for **72** where the dissociation time was prolonged to 120 s. A 10 mM galactose solution was injected at the end of each cycle

for regeneration of the surface. For compound **72**, since the signal did not return to the baseline, the surface was regenerated with a 1 min injection of HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 0.005% polysorbate 20, 3 mM EDTA, pH 7.4), and was reconstituted in running buffer for 10 min. A series of 1 mM galactose, Gal*N*Ac, and Glucose was injected at the beginning, in the middle, and at the end of the ranking experiment as positive and negative controls. After subtraction of the reference cell, the binding signals were determined 10 s before injection end and were corrected for the molecular weight (normalization). All signals for **72** had a positive sign and were not mirrored before fitting. Despite the rather slow binding kinetics, the apparent K_D value could be estimated by fitting the values at 25 s to a simple 1:1 binding model. However, the binding curves did not fit to a standard kinetic model.

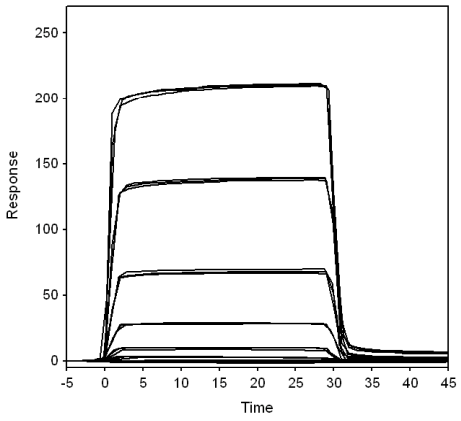
High-resolution screening

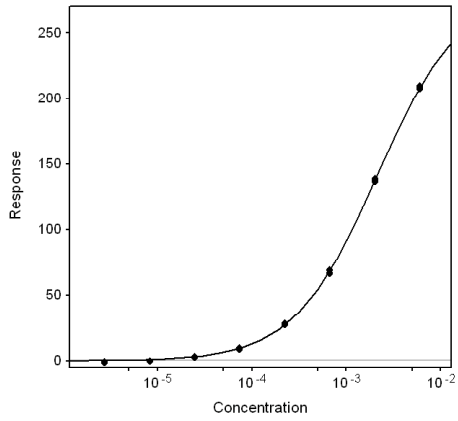
Running buffer with 3% DMSO was used for all high-resolution screening experiments. 200 mM stock solutions in DMSO were diluted with DMSO-free buffer to a compound concentration of 6 mM in 3% DMSO. Threefold dilution series between 6 mM and 2.7 µM (8 concentrations) were prepared in running buffer. All samples were injected as randomized triplicates for 30 s at a flow rate of 50 µl/min, followed by 20 s undisturbed dissociation. No regeneration had to be applied between the cycles. Eight buffer blanks were injected during each experiment for conditioning the surface and for double referencing.[103] Since injection of sugar ligands to immobilized H1-CRD is described to cause negative binding responses,[53] all signals were mirrored by multiplication of each data point per -1. The processed steady state responses were plotted against the concentration and were fitted to a single binding site model.



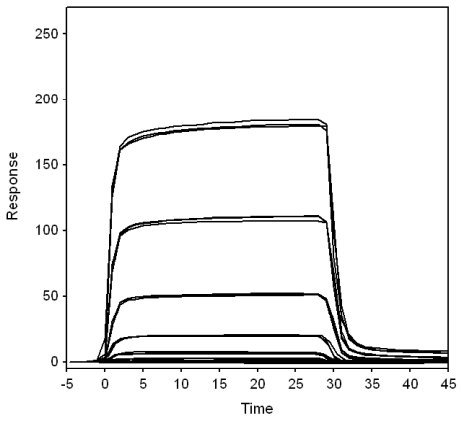


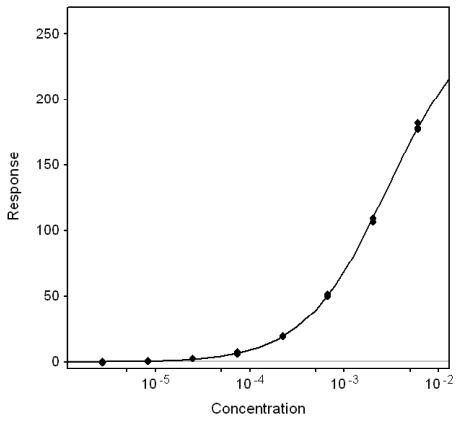
Gal*N*Ac



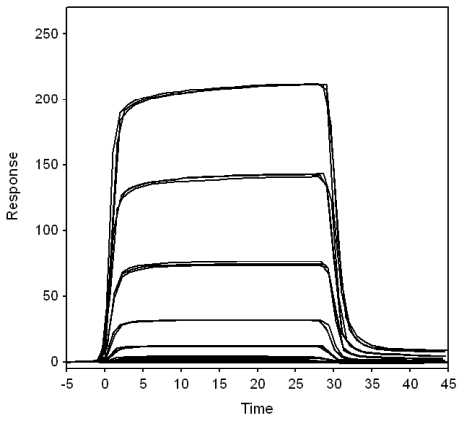


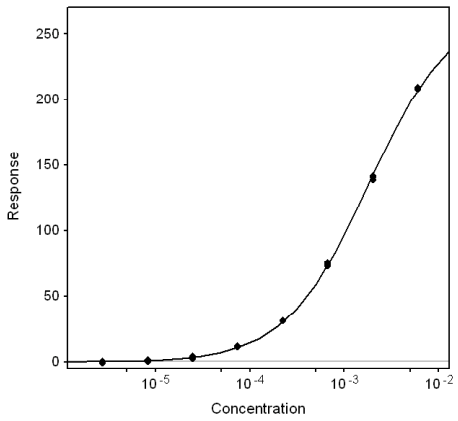
Gal



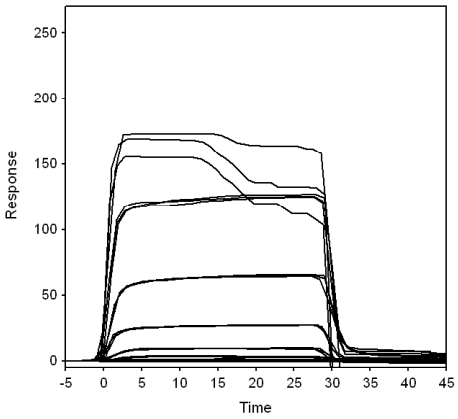


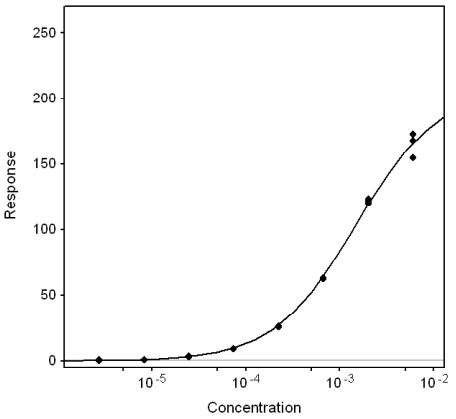
Methyl α-Gal





Methyl β-Gal





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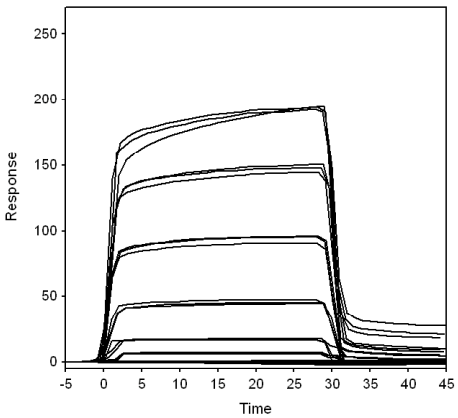
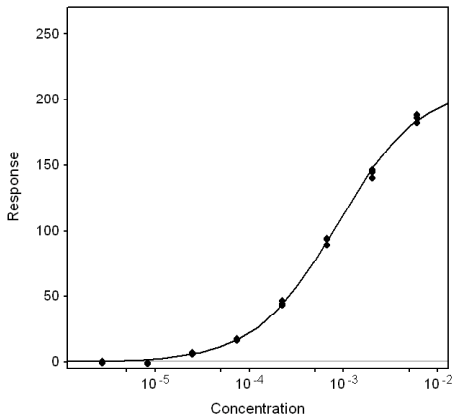
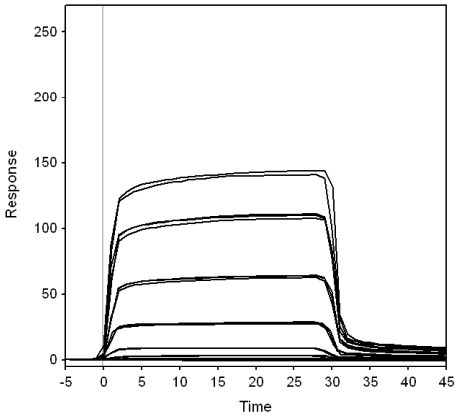
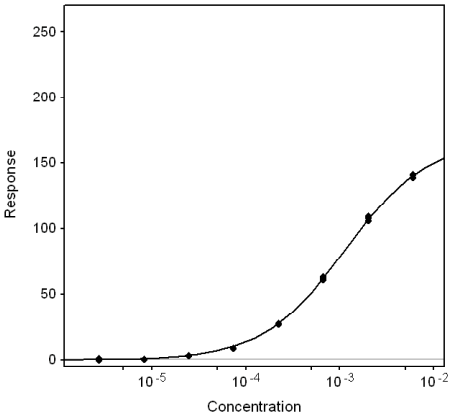


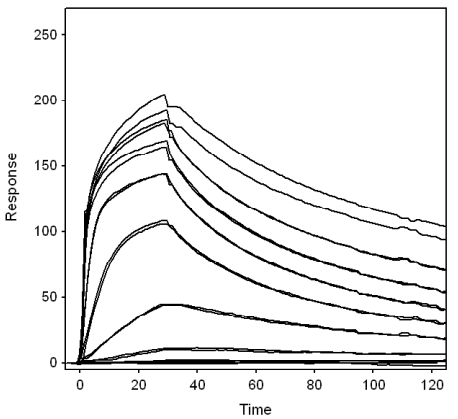


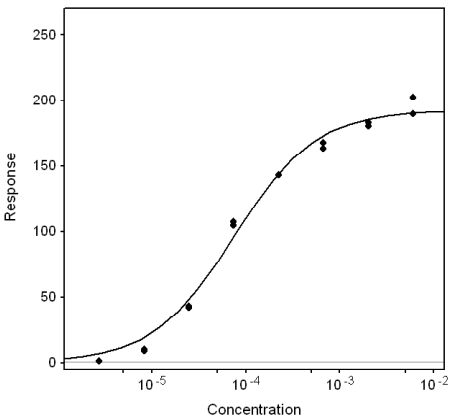
46





39





72
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Curriculum Vitae

Claudia Riva Mahler

Personal Details

Date of birth 12th July 1974.
Place of birth Montevideo, Uruguay.
Nationality Italian and Uruguayan.

Address Birsigstrasse 131,
4054 Basel, Switzerland.
Phone number 061 281 60 79 (home).
076 323 24 65 (mobile).

E-mail claudia.riva_mahler@novartis.com

Education

2006 Doctor (Ph. D.), University of Basel, Institute of Molecular Pharmacy, under the supervision of Prof. Dr. Beat Ernst. Thesis on carbohydrate chemistry: "Targeting the liver via the Asialoglycoprotein receptor: Synthesis of directed compound libraries (in solution and on solid phase) for the H1-CRD receptor and biological evaluation of ligands for better understanding the binding requirements".

2001 Graduate in Pharmaceutical Chemistry ("Química Farmacéutica"), Universidad de la República, Uruguay.

1997 Undergraduate in Chemistry ("Bachiller en Química"), Universidad de la República, Uruguay.

Professional Experience

April 2006 - present Reg CMC documentation manager, drug substance writer at Novartis Pharma AG, Basel, Switzerland.

February 2004 - July 2004 Tutorial of a diploma work of a Pharmacy student at the University of Basel. Synthesis of carbohydrate libraries using 1,3-dipolar-cycloaddition reactions.

November 2001 - March 2005 Assistant for the practical course of third and fourth year of Pharmacy, Institute of Molecular Pharmacy, University of Basel. Solid phase synthesis of peptides and HPLC analysis. Also responsible for the material supply of the course.

August 1999 - July 2000 Traineeship in combinatorial chemistry at the Core Technology Area of Novartis Pharma AG, Basel, Switzerland. Supervised by Dr. W. Brill and Dr. J. Zimmerman.

Publications

Riva-Toniolo C.; Müller S.; Schaub J.; Brill W. K. D. **Catalysis of nucleophilic aromatic substitutions in the 2,6,8-trisubstituted purines and application in the synthesis of combinatorial chemistry**, Molecular diversity, (**2003)**, 6 (1), 43-53.

Brill, W. K. D.; Riva-Toniolo C. **Solid phase synthesis of 2,6,8-trisubstituted purines**, Tetrahedron letters (**2001)**, 42, (37), 6515-6518.

Brill, W. K. D.; Riva-Toniolo C. **The bromination of purines with a charge transfer complex between bromine and lutidine**, Tetrahedron letters (**2001)**, 42, (36), 6279-6282.

Brill, W. K. D.; Riva-Toniolo C.; Müller S. **Catalysis of 2- and 6-substitution reactions of purines on solid phase**, Synlett (**2001)**, (7), 1097-1100.
Riva-Toniolo C.; Müller S.; Schaub J.; Brill W. K. D. **Substitution reactions on C2 and C8 of purines on a solid support.** International Electronic Conference on Synthetic Organic Chemistry (http://www.mdpi.org/ecsoc-5.htm, 5th and 6th Sept. 1-30, 2001 and 2002 and 7th, 8th Nov. 1-30 2003 and 2004 (2004), 807-815.

Awards

September 2006	Above and Beyond Award (Novartis): excellent writing & communication with internal and external sites.
August 2006	Above and Beyond Award (Novartis): organization of several complicated variations. These variations necessitated intense and tenacious follow up with internal and external sites.
February 2005	Best Poster Price Winner at "DoktorandInnen Tag" of the Center of Pharmaceutical Sciences University of Basel-ETH Zurich.

Languages

Spanish	Mother tongue.
English	Fluent.
German	Spoken fluent and written intermediate.
French	Spoken fluent.
Italian	Spoken good knowledge.
Portuguese	Spoken good knowledge.

Congress and seminars

February 2005	"DoktorandInnen Tag" of the Center of Pharmaceutical Sciences University of Basel-ETH Zurich.
September 2004	Key Issues in Drug Discovery and Development: 7 days course from the Center of Pharmaceutical Sciences University of Basel-ETH Zurich.
July 2003	Second Joint French-Swiss in Medicinal Chemistry, Beaune France.
January 2002	5th Swiss Course on Medicinal Chemistry, Leysin-Switzerland.

Extracurricular activities

April 2004 - May 2005	Participated in **WIN** (Women into Industry) project between the University of Basel and Novartis with Dr. E. Francotte as mentor (Unit Head of Separations in Discovery Technologies).
January 2000 - July 2000	Founding member and President of Basel Local Committee of IAESTE (International Association for the Exchange of Students for Technical Experience).
1990 - 1998	Volunteer leader in the YMCA (Young Men Christian Association) leading groups of children from 3 to 15 years old and family's camps.
1994 - 1995	English teacher of first, second and fifth term in Uruguayan high schools.

1990 - 1991 Theater course. Main roles in three plays.